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(54) Title: **USE OF MONO AND BICARBOXYLIC ACID AMIDES FOR THE MANUFACTURE OF A MEDICAMENT ACTIVE AT THE PERIPHERAL CANNABINOID RECEPTOR**

(57) Abstract

The following description concerns the therapeutic use of amidic derivatives of mono and dicarboxylic acids with aminoalcohols or aminoethers in the manufacture of a medicament for the treatment of diseases connected with the modulation of the cannabinoid peripheral receptor, said amidic derivatives being selectively active on said receptor.

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USE OF MONO AND BICARBOXYLIC ACID AMIDES FOR THE MANUFACTURE OF A MEDICAMENT ACTIVE AT THE PERIPHERAL CANNABINOID RECEPTOR

FIELD OF THE INVENTION

5 The present invention relates to the therapeutic use of amidic derivatives of mono and dicarboxylic acids with substituted aminoalcohols or aminoethers, in the treatment of diseases connected with the modulation of the cannabinoid peripheral receptor.

PRIOR ART DISCLOSURE

10 Cannabinoids are a specific class of psychoactive compounds present in Indian cannabis (*Cannabis sativa*), including about 60 different molecules, the most representative being cannabinol, cannabidiol and several isomers of tetrahydrocannabinol. Knowledge of the therapeutic activity of cannabis dates back to the ancient dynasties in China, 15 where, already 5.000 years ago, cannabis was used for the treatment of asthma, migraine and some gynaecologic disorders. Said use later became so established that about in 1850 cannabis extracts were included in the US Pharmacopoeia and remained therein until 1947.

20 Cannabinoids are able to cause different effects at the level of various systems and/or organs; the most important effects occur on the central nervous system and on the cardiovascular system. In fact, they are able to affect mood, memory, motor coordination and cognition, and they increase heart rate and variate the systemic arterial pressure. Furthermore, it is well known the capability of cannabinoids to reduce 25 intraocular pressure and to affect the respiratory and endocrine systems (L.E. Hollister, Health Aspects of Cannabis, Pharmacological Reviews, 38, 1-20, 1986). More recently, it was found that they

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suppress the cellular and humoral immune response and have antiinflammatory properties (A.W. Wirth et al., Antiinflammatory Properties of Cannabichromene, *Life Science*, 26, 1991-1995, 1980).

5 However, the therapeutic use of cannabis is controversial both due to its relevant psychoactive effects, causing dependence and addiction, and to the manifold side effects that have not yet been completely clarified (L.E. Hollister, 1986, reference cited).

10 Notwithstanding the well established use of cannabis over the centuries, the mechanism of the effects of cannabinoids has been unknown until very recently. It was only in 1990 that Matsuda and

collaborators identified and cloned a cannabinoid receptor belonging to the G-protein-coupled family of receptors; CB1 is coupled to G1 to inhibit adenilate cyclase activity and to a partussis-sensitive G protein to regulate Ca^{2+} currents. Said receptor was found to be

15 mainly located in the brain, in neural cell lines and only to a lesser extent at a peripheral level; therefore, in view of its localization, it was called Central Receptor (CB1) (Matsuda et al., Structure of a cannabinoid receptor and functional expression of the cloned cDNA, *Nature*, 346: 561-564, 1990). The discovery of a receptor

20 led to assume the existence of a specific endogenous ligand.

In fact, subsequent research led to the isolation from pig brain of a substance able to exert an agonist action, i.e. capable of binding to the cannabinoid central receptor in a competitive way. Said substance was identified by structural investigation and by comparison with the

25 synthetic product and was found to be an amidic derivative of arachidonic acid, and more particularly arachidonylethanolamide, later called anandamide. The pharmacological characterization of said

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molecule provided evidence that anandamide possesses a profile of activity which is similar to, though less potent than, $\Delta 9$ -THC (tetrahydrocannabinol with a double bond in position 9), and is capable of mimicking the psychoactive effects thereof. Said evidences 5 led to the conclusion that anandamide is the endogenous ligand of the cannabinoid central receptor (C.C. Felder et al., Anandamide, an Endogenous Cannabimimetic Eicosanoid. Binds to the Cloned Human Cannabinoid Receptor and Stimulates Receptor-mediated Signal Transduction. PNAS. 90. 7656-7660. 1993; P.B. Smith et al., The 10 Pharmacological Activity of Anandamide, a Putative Endogenous Cannabinoid, in Mice. J. PET. 270, 219-227, 1994).

Subsequent researches brought about the individuation of substances binding to CB1 receptor; said substances, which may be grouped into a class of amidic compounds, were denominated anandamides by the authors 15 (L. Hanus et al., Two New Unsaturated Fatty Acids Ethanolamides in the Brain that Bind to the Cannabinoid Receptor. J.Med.Chem.. 36, 3032-3034, 1993). The discovery that the ethanolamide of arachidonic acid, but not the ethanolamide of another biologically significant acid and anyway endogenously present in the brain such as palmitic acid, can 20 functionally activate CB1 central receptor, brought about the subsequent identification of other amides of ethanolamine with highly unsaturated fatty acids, which have an affinity to CB1 receptor.

The multiplicity of effects of cannabinoids and CB1 receptor peculiar distribution led to assume the existence of differentiated receptor 25 sites. In fact, a second different receptor for cannabinoids, called Peripheral Receptor (CX5 or CB2), was cloned. Being present in the spleen and macrophages/monocytes but absent at a central level, said

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receptor was regarded as responsible for mediating the cannabinoid-induced non-psychoactive effects (S. Munro et al., Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365, 61-65, 1993). In this regard, the Δ 9-THC ability to induce immunosuppressive effects was proved. Recent experimental results showed that Δ 9-THC can cause alteration in the macrophagic function. In fact, the exposure to Δ 9-THC decreases the cytolytic activity of activated macrophages, measured as TNF- α synthesis, release and cytotoxicity. Moreover, since macrophages release several cytolytic molecules, other than TNF- α , they were supposed to be a target for Δ 9-THC (K. Fischer- Stenger et al.. Δ 9-Tetrahydrocannabinol Inhibition of Tumor Necrosis Factor- α : Suppression of Post-translational Events. *J. PET*, 267, 1558- 1565, 1993). All the above evidences and the preferential massive localization of CB2 receptor at the immune system level prove that said receptor plays a specific role in mediating the immune and antiinflammatory response to stimuli of a different nature, the bacterial and viral ones included.

It was also demonstrated that anandamide, the endogenous ligand of CB1 central receptor, can bind to CB2 receptor with an affinity about 30 times lower than to the central receptor; this suggests the existence of another endogenous ligand for said receptor, so far unidentified (L.L. Iversen. Medical uses of marijuana?. *Nature*, 365, 12-13, 1993).

As already mentioned, the therapeutic use of cannabinoids as analgesic, anti-emetic, anticonvulsant, spasmolytic and antiglaucoma agents, and, as more recently found, as anti-inflammatory agents, is inadvisable because of the cannabinoid-induced untoward side effects and psychoactive effects, as well as addiction and pharmacological

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dependence (W.L. Dewey, Cannabinoid Pharmacology, 38 (2), 151-178, 1986).

Some compounds, able to act as agonists indiscriminately on both cannabinoid receptors, have been recently developed: for example, it 5 is known the use of dihydropyrrole-(1,2,3-d,e)-1,4-benzooxazine derivatives in the treatment of glaucoma (USP 5.112.820, Derwent abstract) and the use of diphenyl-pyrazole derivatives as immunomodulators or psychotropic agents in the treatment of various neuropathies, migraine, epilepsy, glaucoma etc. (European Patent 10 Application EP 576 357, Derwent abstract). However said compounds, active on both CB1 central and CB2 peripheral receptors, may cause significant psychoactive effects, as well as addiction and habit.

In the light of the discovery that cannabinoids act through a receptorial mechanism and, in particular, on receptors able to mediate 15 different functional effects, and that the two central and peripheral receptors, present little homology, it is evident the importance of developing a class of drugs acting selectively on the receptor subtype and not indiscriminately on both receptors, as is the case of natural and synthetic cannabinoids.

20 The investigations conducted to date on the pharmacological effects mediated by cannabinoid receptors show that the non-psychoactive effects of Cannabis derivatives are mediated by CB2 peripheral receptor. Furthermore, the CB2 receptor localization proves that said non-psychoactive effects, i.e. the effects on the immune system, the 25 anti-inflammatory, myorelaxant and antinociceptive effects, as well as the effects on pressure systems, are mediated by said receptor.

The above evidences clearly show that it would be extremely important,

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from a therapeutic viewpoint, to obtain compounds capable of selectively acting on the cannabinoid peripheral receptor and, therefore, on the diseases connected with the modulation of said receptor, without causing psychoactive effects at a central level and the relevant side effects related to such action, such as habit and 5 addiction.

The Applicant has recently found a class of N-acyl derivatives active on the modulation of mast cell degranulation processes, which act according to a local antagonist endogenous regulation mechanism meant 10 to control the mast cell degranulation induced by neurogenic and immunogenic supramaximal stimuli (ALIA = Autacoid Local Inflammation Antagonism). Said compounds can be advantageously used in the treatment of neurogenic and/or immunogenic autoimmune diseases, in particular multiple sclerosis and psoriasis, as disclosed in European 15 Patent Applications EP 0 550 006 and EP 0 550 008. As known, the autoaggressive pathological phenomena of autoimmune diseases occur through local tissue injury, where specific immunocompetent cells, among which mast cells, play a central etiopathogenic role.

In fact, mast cells are a cellular population diffused in the tissues 20 which, becoming active in situ, causes biological inflammatory events with liberation of several highly cytotoxic chemical mediators, responsible for local tissue injury. Mast cell activity is controlled in an agonist sense by neuro and immunomediated activating systems, antagonized by inhibitory systems acting through general circuits, 25 such as e.g. the corticosteroid hormones.

The Applicant found that a class of N-acyl-alkanolamides, in particular N-acyl-monoethanolamides and N-acyl-diethanolamides, is

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able to act on mast cells as a local antagonist system according to a mechanism of autacoid type; therefore, such compounds can be conveniently used in the treatment of diseases connected with acute or chronic inflammatory events, such as for example autoimmune diseases.

5 To said class of compounds belongs also N-(2-hydroxyethyl)-hexadecanamide, or N-palmitoyl-ethanolamide (N-PEA), a compound whose activity was discovered accidentally in the '50s, following the identification of a generic cytoprotective activity of a lipidic excipient of an antirheumatic drug, which contained said compound. The 10 pharmacological profile of such compound was later studied in experimental models of injury caused by various agents: the ability to increase the animal resistance to various bacterial toxins led to the subsequent pharmaceutical development of said compound. In fact, a pharmaceutical product was developed in Czechoslovakia in the form of 15 tablets, suitable for preventing infections of the respiratory tract. Therefore, the Applicant found that the activity exerted by N-PEA and by the wide class of N-acyl derivatives described in the aforementioned patent applications does not limit to a generic and modest cytoprotective activity, but said class of compounds plays a 20 specific and important role in local inhibitory modulation of mast cell degranulation.

Such an activity allows the use of the compounds belonging to said class as drugs, exerting not only a generic cytoprotective action, but specifically a modulation of mast cell degranulation and, therefore, 25 an inhibitory action on the autoaggressive effects in autoimmune diseases and on the cytotoxic and lesive effects of diseases of different etiology, connected with inflammations. Said compounds are,

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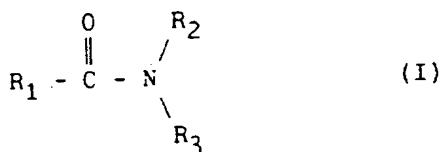
therefore, capable of inhibiting the uncontrolled release of preformed mast cell granules, containing several proinflammatory mediators and, in particular, preformed granules containing Tumour Necrosis Factor (TNF- α), a highly cytotoxic cytokine involved in the autoaggressive 5 autoimmunity process (R. Toms et al., J. Neuroimmunology, 30, 169-177, 1990; P.G. Kruger et al., Acta Neurol.Scand., 81, 331-336, 1990), as well as in lesive processes at the level of different tissues and organs.

Furthermore, the Italian Patent Application No. MI 94 A 000523, in the 10 name of the same Applicant, discloses the use of N-acyl derivatives of aminoalcohols with mono and bicarboxylic acids in the therapeutic prevention and treatment of diseases associated with supramaximal and prolonged stimulation of the receptors of excitatory aminoacids (EAA), in particular of the N-methyl-D-aspartate (NMDA) receptor.

15 SUMMARY OF THE INVENTION

The Applicant has found that amides of mono and bicarboxylic aliphatic acids with aminoalcohols and aminoethers are able to bind selectively to cannabinoid peripheral receptor CB2 and to activate functionally the same.

20 Said amides have formula (I):



where R_2 and R_3 may belong to one of the following classes:

A) R_2 is the residue of a linear or branched hydroxylalkyl containing 1 to 20 carbon atoms, optionally substituted with one or more phenyl groups;

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R₃ is H, CH₃ or = R₂;

B) R₂ is the residue of an alkylene-hydroxyphenyl, the aromatic ring being optionally substituted with one or more -OH and/or -OCH₃ groups, and the linear or branched alkylene chain containing 1 to 20 carbon atoms;

5

R₃ is H, CH₃ or = R₂;

10

C) R₂ and R₃, with the nitrogen atom to which said two substituents are bound, form the residue of a cyclic aminoether containing 5 to 7 carbon atoms, optionally substituted with linear or branched alkyl groups.

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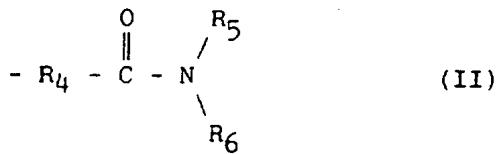
In the aforesaid classes, the alcoholic function -OH can be optionally functionalized to give -OX, where X can be an alkyl, an acyl, an O-phosphate, the amiacyl of a bicarboxylic acid, an alkyl-sulphonate, a sulphate, a dialkylaminoacyl or an aminoacyl, X being optionally salified with monovalent or bivalent inorganic ions.

The substituent R₁ can be:

1) a linear or branched hydrocarbon radical containing 9 to 23 carbon atoms, preferably 11 to 17, either saturated or containing one double bond, optionally substituted with one or more -OH groups;

20

2) a group of formula (II):



where R₄ is a linear or branched hydrocarbon radical containing 8 to 22 carbon atoms, preferably 10 to 16, either saturated or containing a double bond, optionally substituted with one or more -OH groups;

R₅ and R₆ are defined as R₂ and R₃ respectively.

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Therefore, the object of the present invention is the use of said derivatives and of more soluble and/or slow-releasing derivatives thereof as the active principle in the preparation of pharmaceutical compositions for the treatment of diseases of mammals connected with 5 the modulation of cannabinoid peripheral receptor.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the specific hybridization of cannabinoid peripheral receptor amplified by Polymerase Chain Reaction (PCR) in A) rat spleen, B) rat peritoneal mast cell cultures and C) RBL-2H3 cell 10 cultures.

Figure 2 illustrates the specific mRNA-CB2 cannabinoid peripheral receptor detected by in situ hybridisation in Cerebellar granule cells (Fig. 2A) and Cerebellum (Fig. 2B).

DETAILED DESCRIPTION OF THE INVENTION

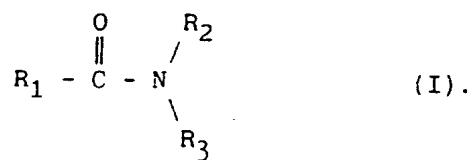
15 The characteristics and advantages of the mono and dicarboxylic acids amides with aminoalcohols or aminoethers, selectively active on the cannabinoid peripheral receptor and suitable for the treatment of diseases connected with the modulation of said receptor, according to the present invention, will be described in details hereinafter.

20 The Applicant has surprisingly found that the activity of N-PEA and of the amides of the invention does not limit to a generic cytoprotective action or to an inhibitory action on mast cell degranulation or to a protective effect against excitatory amino acids cytotoxicity in neural cells, as known in the prior art, but said compounds play an 25 important and specific role in the selective activation of cannabinoid peripheral receptor.

The mono and dicarboxylic acids amides with aminoalcohols or

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aminoethers, active in the treatment of diseases connected with the modulation of cannabinoid peripheral receptor (CB2) or profiting by the activation of said receptor with a consequent negative modulation of cytotoxic and proinflammatory phenomena, according to the present invention, are defined by formula (I):



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When R_2 and R_3 belong to class (A), R_2 preferably forms, with the nitrogen atom to which it is linked, the residue of monoethanolamine, diethanolamine, 2-hydroxy-propylamine or di-(2-hydroxy-propyl)-amine; in these two last cases, the corresponding compound (I) may be 5 optically active or racemic.

When alcoholic residues R_2 and R_3 belong to class (B), R_2 preferably forms, with the nitrogen atom to which it is linked, the residue of tyramine or 4-hydroxy-3-methoxy-benzylamine, both optically active and racemic.

10 When alcoholic residues R_2 and/or R_3 belong to class (C), R_2 and R_3 preferably form, with the nitrogen atom to which they are linked, morpholine residue.

The hydroxyl groups $-OH$ can be optionally functionalized in order to give $-OX$, where X can be an alkyl, preferably methyl or ethyl; an 15 acyl, preferably $-CO-CH_3$ or $-CO-Ph$; an O-phosphate, preferably $-PO_3H_2$ or $-PO_2H-O-CH_2-CH(OH)-CH_2-OH$; the amiacyl of a bicarboxylic acid, preferably $-CH_2-CH_2-COOH$ or $-CO-(CH_2)_3-COOH$; an alkyl-sulphonate, preferably $-SO_2-CH_3$, $-SO_2-C_6H_5$ or $-SO_2-C_6H_4-CH_3$; a sulphate; a dialkylaminoacyl, preferably $-CO-CH_2-N(CH_3)_2$; an aminoacyl, preferably 20 $-CO-CH(W)-NH_2$, wherein W is the lateral chain of a natural aminoacid; X being optionally salified with monovalent or bivalent inorganic ions, and preferably K, Na, Mg or Ca.

The function of the group X is to increase the solubility in water and/or modify the pharmacokinetic properties of the compound, giving 25 pro-drugs. When R_1 belongs to class (1), it forms, with the adjacent carbonyl, the acyl of a monocarboxylic acid, and preferably of lauric, myristic, palmitic, stearic, palmitoleic and oleic acids, or the

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homologues thereof substituted with hydroxyl groups, such as e.g. ω -hydroxypalmitic acid.

When R_1 belongs to class (2), R_4 forms, with the two adjacent carbonyls, the acyl of a bicarboxylic acid, and preferably of 5 traumatic acid.

The products of the present invention may be prepared according to various known procedures, and preferably by high-temperature melting of the alkanolamine salt with the carboxylic acid, with formation of the relevant alkanolamide, or by acylation to the nitrogen atom of 10 alkanolamine with a suitable carboxylic acid activated derivative, or by activation of the carboxyl of the acid with alkylchloroformate followed by aminolysis with alkanolamine.

Some examples of preparation of the amides of mono and bicarboxylic acids according to the present invention are reported hereinbelow for 15 illustrative but not limitative purposes.

EXAMPLE 1: Preparation of N-palmitoyl-[(R)-2-amino-2-phenylethanol]

(R)-2-amino-2-phenylethanol (1.37 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous 20 tetrahydrofuran (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from tert-butyl-methyl-

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ether (30 ml). The product was separated by filtration, washed twice with tert-butyl-methyl-ether (5 ml), and dried in high vacuo.

The reaction yield was 88% approx.

The physico-chemical properties of N-palmitoyl-[(R)-2-amino-2-

5 phenylethanol] were as follows:

physical state	white crystalline powder
molecular formula	C ₂₄ H ₄₁ NO ₂
molecular weight	375.59
elemental analysis	C=76.75%; H=11.00%; N=3.73%; O=8.52%
10 solubility in organic	
solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol
solubility in water	scarcely soluble
melting point	93-95°C
15 TLC	eluent: toluene-chloroform-acetone. 40/25/35; Rf=0.54

EXAMPLE 2: Preparation of N-palmitoyl-[(S)-2-amino-2-phenylethanol]

(S)-2-amino-2-phenylethanol (1.37 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 20 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous tetrahydrofuran (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness. The crude residue was 25 taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with water (15 ml). combined, dehydrated with anhydrous sodium sulphate and evaporated

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under vacuum. The residue was crystallized from tert-butyl-methyl-ether (30 ml). The product was separated by filtration, washed twice with tert-butyl-methyl-ether (5 ml) and dried in high vacuo.

The reaction yield was 91% approx.

5 The physico-chemical properties of N-palmitoyl-[(S)-2-amino-2-phenylethanol] were as follows:

physical state	white crystalline powder
molecular formula	C ₂₄ H ₄₁ NO ₂
molecular weight	375.59
10 elemental analysis	C=76.75%; H=11.00%; N=3.73%; O=8.52%
solubility in organic	
solvents	>10 mg/ml in DMSO;
	>10 mg/ml in ethanol
solubility in water	scarcely soluble
15 melting point	92-94°C
TLC	eluent: toluene/chloroform/acetone, 40:25:35; Rf=0.54

EXAMPLE 3: Preparation of N-(2-hydroxy-2-phenylethyl)-palmitoylamide

2-amino-1-phenylethanol (1.37 g; 10 mmol) and triethylamine (1.13 g; 20 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous tetrahydrofuran (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The 25 resulting suspension was evaporated to dryness. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with water (15 ml),

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combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold ethanol (30 ml). The product was separated by filtration, washed twice with cold ethanol (5 ml) and dried in high vacuo.

5 The reaction yield was 93% approx.

The physico-chemical properties of N-(2-hydroxy-2-phenylethyl)-palmitoylamide were as follows:

	physical state	white crystalline powder
	molecular formula	C ₂₄ H ₄₁ NO ₂
10	molecular weight	375.59
	elemental analysis	C=76.75%; H=11.00%; N=3.73%; O=8.52%
	solubility in organic solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol
15	solubility in water	scarcely soluble
	melting point	86-88°C
	TLC	eluent: toluene-chloroform-acetone. 40/25/35; Rf=0.70

EXAMPLE 4: Preparation of N-palmitoyl-[(1S,2S)-(+)-2-amino-1-phenyl-

20 1,3-propanediol

(1S,2S)-(+)-2-amino-1-phenyl-1,3-propanediol (1.67 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous dimethylformamide (50 ml) at 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous dimethylformamide (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was

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evaporated to dryness under vacuum. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum.

5 The residue was crystallized from cold ethanol 95° (30 ml). The product was separated by filtration, washed twice with cold ethanol 95° (5 ml) and dried in high vacuo.

The reaction yield was 89% approx.

The physico-chemical properties of N-palmitoyl-[(1S,2S)-(+)-2-amino-1-phenyl-1,3-propanediol] were as follows:

physical state white crystalline powder

molecular formula C₂₅H₄₃NO₃

molecular weight 405.62

elemental analysis C=74.03%; H=10.68%; N=3.45%; O=11.83%

15 solubility in organic

solvents >10 mg/ml in DMSO;

>10 mg/ml in ethanol

solubility in water scarcely soluble

melting point 81-83°C

20 TLC eluent: toluene-ethanol-acetic acid,
65/30/5; Rf=0.72

EXAMPLE 5: Preparation of N-palmitoyl-[(1S,2R)-(+)-norephedrine]

(1S,2R)-(+)-norephedrine (1.51 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous chloroform (50 ml) at 0°C. A 25 solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous chloroform (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C

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for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness under vacuum. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with HCl 1N (15 ml) and 5 twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold acetonitrile (30 ml). The product was separated by filtration, washed twice with cold acetonitrile (5 ml) and dried in high vacuo.

10 The reaction yield was 93% approx.

The physico-chemical properties of N-palmitoyl-[(1S,2R)-(+)-norephedrine] were as follows:

physical state	white crystalline powder
molecular formula	C ₂₅ H ₄₃ NO ₂
15 molecular weight	389.62
elemental analysis	C=77.07%; H=11.12%; N=3.59%; O=8.21%
solubility in organic solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol
20 solubility in water	scarcely soluble
melting point	83-85°C
TLC	eluent: toluene-ethanol-acetic acid. 65/30/5; Rf=0.75

EXAMPLE 6: Preparation of N-lauroyl-[(1S,2R)-(+)-norephedrine]

25 (1S,2R)-(+)-norephedrine (1.51 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous chloroform (50 ml) at 0°C. A solution of lauroyl chloride (2.19 g; 10 mmol) in anhydrous chloroform

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(20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was washed twice with HCl 1N (20 ml) and twice with water (15 ml). The aqueous phases were extracted twice with chloroform (15 ml). The organic phases were combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold acetonitrile (30 ml). The product was separated by filtration, washed twice with cold acetonitrile (5 ml), and dried in high vacuo.

The reaction yield was 89% approx.

The physico-chemical properties of N-lauroyl-[(1S,2R)-(+)-norephedrine] were as follows:

physical state	white crystalline powder
15 molecular formula	C ₂₁ H ₃₅ NO ₂
molecular weight	333.51
elemental analysis	C=75.63%; H=10.58%; N=4.20%; O=9.59%
solubility in organic solvents	>10 mg/ml in DMSO;
20	>10 mg/ml in ethanol
solubility in water	scarcely soluble
melting point	72-74°C
TLC	eluent: toluene-ethanol-acetic acid, 65/30/5; R _f =0.70

25 EXAMPLE 7: Preparation of N-palmitoyl-[(1R,2S)-(-)-norephedrine]

(1R,2S)-(-)-norephedrine (1.51 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous chloroform (50 ml) at 0°C. A

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solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous chloroform (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness under vacuum. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with HCl 1N (15 ml) and twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold acetonitrile (30 ml). The product was separated by filtration, washed twice with cold acetonitrile (5 ml) and dried in high vacuo.

The reaction yield was 90% approx.

The physico-chemical properties of N-palmitoyl-[(1R,2S)-(-)-15 norephedrine] were as follows:

physical state	white crystalline powder
molecular formula	C ₂₅ H ₄₃ NO ₂
molecular weight	389.62
elemental analysis	C=77.07%; H=11.12%; N=3.59%; O=8.21%
20 solubility in organic	
solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol
solubility in water	scarcely soluble
melting point	82-84°C
25 TLC	eluent: toluene-ethanol-acetic acid. 65/30/5; R _f =0.56

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EXAMPLE 8: Preparation of N-lauroyl-[(1R,2S)-(-)-norephedrine]

(1R,2S)-(-)-norephedrine (1.51 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous chloroform (50 ml) at 0°C. A solution of lauroyl chloride (2.19 g; 10 mmol) in anhydrous chloroform 5 (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was washed twice with HCl 1N (20 ml) and twice with water (15 ml). The aqueous phases were extracted twice with chloroform (15 ml). The 10 organic phases were combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold acetonitrile (30 ml). The product was separated by filtration, washed twice with cold acetonitrile (5 ml) and dried in high vacuo.

15 The reaction yield was 89% approx.

The physico-chemical properties of N-lauroyl-[(1R,2S)-(-)-norephedrine] were as follows:

physical state	white crystalline powder
molecular formula	C ₂₁ H ₃₅ NO ₂
20 molecular weight	333.51
elemental analysis	C=75.63%; H=10.58%; N=4.20%; O=9.59%
solubility in organic	
solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol
25 solubility in water	scarcely soluble
melting point	72-74°C
TLC	eluent: toluene-ethanol-acetic acid.

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65/30/5; Rf=0.70

EXAMPLE 9: Preparation of N-palmitoyl-[(S)-(-)-2-amino-3-phenyl-1-propanol]

(S)-(-)-2-amino-3-phenyl-1-propanol (1.51 g; 10 mmol) and 5 triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous tetrahydrofuran (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room 10 temperature for additional 5 hrs. The resulting suspension was evaporated to dryness under vacuum. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with HCl 1N (15 ml) and twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and 15 evaporated under vacuum. The residue was crystallized from cold ethanol (30 ml). The product was separated by filtration, washed twice with cold ethanol (5 ml) and dried in high vacuo.

The reaction yield was 94% approx.

The physico-chemical properties of N-palmitoyl-[(S)-(-)-2-amino-3-phenyl-1-propanol] were as follows:

physical state	white crystalline powder
molecular formula	C ₂₅ H ₄₃ NO ₂
molecular weight	389.62
elemental analysis	C=77.07%; H=11.12%; N=3.59%; O=8.21%
25 solubility in organic solvents	>10 mg/ml in CHCl ₃ ;
solubility in water	scarcely soluble

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melting point 96-98°C

TLC eluent: toluene-ethanol-acetic acid.
65/30/5; Rf=0.70

EXAMPLE 10: Preparation of N-palmitoyl-[(R)-(+)-2-amino-3-phenyl-1-5 propanol]

(R)-(+)-2-amino-3-phenyl-1-propanol (1.51 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous tetrahydrofuran (20 ml) was added dropwise. 10 over a period of 30 min. under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness under vacuum. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases 15 were washed twice with HCl 1N (15 ml) and twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold ethanol (30 ml). The product was separated by filtration, washed twice with cold ethanol (5 ml) and dried in high vacuo. 20 The reaction yield was 89% approx.

The physico-chemical properties of N-palmitoyl-[(R)-(+)-2-amino-3-phenyl-1-propanol] were as follows:

physical state white crystalline powder

molecular formula C₂₅H₄₃NO₂

25 molecular weight 389.62

elemental analysis C=77.07%; H=11.12%; N=3.59%; O=8.21%

solubility in organic

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solvents	>10 mg/ml in CHCl ₃ :
solubility in water	scarcely soluble
melting point	96-98°C
TLC	eluent: toluene-ethanol-acetic acid.
5	65/30/5; Rf=0.70

EXAMPLE 11: Preparation of N-(p-hydroxyphenylethyl)-lauroylamide

Tyramine (1.37 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in isopropanol (50 ml) at -5°C. A solution of lauroyl chloride (2.19 g; 10 mmol) in anhydrous chloroform (20 ml) was added 10 dropwise, over a period of 30 min. under continuous stirring at 0°C. The resulting mixture was evaporated to dryness and the residue was taken up with ethyl acetate (70 ml). The resulting solution was washed twice with HCl 1N (20 ml) and twice with water (15 ml). The aqueous phases were extracted twice with ethyl acetate (15 ml). The organic 15 phases were combined, decolourized with bone charcoal, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from tert-butyl-methyl-ether (30 ml). The product was separated by filtration, washed twice with cold tert-butyl-methyl-ether (5 ml) and dried in high vacuo.

20 The reaction yield was 89% approx.

The physico-chemical properties of N-(p-hydroxyphenylethyl)-lauroylamide were as follows:

physical state	white crystalline powder
molecular formula	C ₂₀ H ₃₃ NO ₂
25 molecular weight	319.49
elemental analysis	C=75.19%; H=10.41%; N=4.38%; O=10.02%

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solubility in organic

solvents >10 mg/ml in DMSO:

>10 mg/ml in ethanol

solubility in water scarcely soluble

5 melting point 93-95°C

EXAMPLE 12: Preparation of N-(3-hydroxypropyl)-lauroylamide

3-amino-1-propanol (0.75 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous ethyl acetate (50 ml) at 0°C. A solution of lauroyl chloride (2.19 g; 10 mmol) in anhydrous ethyl acetate (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was extracted three times with water (20 ml) and the aqueous phases were extracted again twice with ethyl acetate (20 ml). The organic phases were combined, dehydrated with anhydrous sodium sulphate and concentrated to a volume of 20 ml approx. The solution was allowed to crystallize at 0°C and the crystalline fraction was separated by filtration, washed twice with ethyl acetate (5 ml) and dried in high vacuo.

The reaction yield was 95% approx.

The physico-chemical properties of N-(3-hydroxypropyl)-lauroyl amide were as follows:

25 physical state white crystalline powder

molecular formula C₁₅H₂₁NO₃

molecular weight 257.42

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elemental analysis C=69.99%; H=12.14%; N=5.44%;
O=12.43%

solubility in organic

solvents >10 mg/ml in DMSO;

5 >10 mg/ml in ethanol

solubility in water scarcely soluble

melting point 77-79°C

TLC eluent: toluene-ethanol-acetic acid.

65/30/5; Rf=0.52

10 EXAMPLE 13: Preparation of N,N'-bis-(3-hydroxypropyl)trans-2-dodecenediamide

Traumatic acid (2.28 g; 10 mmol) was solubilized in anhydrous dimethylformamide (40 ml) at 0°C with pyridine (2 ml).

N-hydroxysuccinimide (2.53 g; 22 mmol) and dicyclohexylcarbodiimide

15 (4.12 g; 20 mmol) were added to the above solution and the resulting mixture was stirred at 0°C for 2 hrs. The urea formed was separated by filtration and discarded. The solution containing the succinimide ester was stirred again at 0°C and added with 3-amino-1-propanol (2.25 g; 30 mmol). The resulting mixture was stirred overnight at room 20 temperature and evaporated to dryness under vacuum. The residue was crystallized twice from water (60 ml). The crystalline fraction was separated by filtration, washed twice with water (5 ml) and dried in high vacuum.

The reaction yield was 92% approx.

25 The physico-chemical properties of N,N'-bis(3-hydroxypropyl)-trans-2-dodecenediamide were as follows:

physical state

white crystalline powder

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molecular formula	$C_{18}H_{34}N_2O_4$
molecular weight	342.48
elemental analysis	C=63.13%; H=10.01%; N=8.18%; O=18.69%
solubility in organic	
5 solvents	>5 mg/ml in DMSO;
solubility in water	scarcely soluble
melting point	142-143°C
TLC	eluent: chloroform-methanol-water- NH_3 (28%). 80/25/2/1; R_f =0.59

10 EXAMPLE 14: Preparation of N-methyl-N-(2-hydroxyethyl)-palmitoylamide

A mixture of palmitic acid (2.57 g; 10 mmol) and 2-(methylamino)-ethanol (1.13 g; 15 mmol) was fed to a flask provided with reflux condenser and heated on an oil bath to 160°C for 6 hrs. The mixture was cooled, solubilized in ethyl acetate (100 ml) and extracted twice with NaOH 0.1N (20 ml), twice with HCl 0.1N (20 ml) and twice with H_2O (20 ml). The aqueous phases were washed twice with ethyl acetate (10 ml). The organic phases were combined, dehydrated with sodium sulphate and evaporated to dryness. The residue was purified by silica gel column chromatography eluting with chloroform-methanol, 85/15. The fractions containing the product were combined and evaporated to dryness. The residue was dried in high vacuo.

The reaction yield was 80% approx.

The physico-chemical properties of N-methyl-N-(2-hydroxyethyl)-palmitoylamide were as follows:

25 physical state	white amorphous powder
molecular formula	$C_{19}H_{39}NO_2$

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	molecular weight	313.52
	elemental analysis	C=72.79%; H=12.54%; N=4.47%; O=10.20%
	solubility in organic	
5	solvents	>10 mg/ml in DMSO; >10 mg/ml in n-octanol
	solubility in water	scarcely soluble
	melting point	-
	TLC	eluent: chloroform-methanol-water-
10		NH ₃ (28%). 80/25/2/1; Rf=0.80

EXAMPLE 15: Preparation of 2-(N-palmitoylamino)-1,3-propandiol

2-amino-1,3-propanediol (0.91 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 0°C. A solution of palmitoyl chloride (2.74 g; 10 mmol) in anhydrous 15 tetrahydrofuran (20 ml) was added dropwise, over a period of 30 min. under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness. The crude residue was taken up with water (20 ml) and extracted twice with ethyl acetate (20 ml). The organic phases were washed twice with water (15 ml). 20 combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from cold ethanol (30 ml); the product was separated by filtration, washed twice with ethanol (5 ml) and dried in high vacuo.

25 The reaction yield was 89% approx.

The physico-chemical properties of 2-(N-palmitoylamino)-1,3-propandiol were as follows:

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physical state	white crystalline powder
molecular formula	C ₂₉ H ₃₉ NO ₃
molecular weight	329.52
elemental analysis	C=69.25%; H=11.93%; N=4.25%;
5	O=14.57%
solubility in organic	
solvents	>5 mg/ml in DMSO; >5 mg/ml in ethanol
solubility in water	scarcely soluble
10 melting point	124.5-126.5°C
TLC	eluent: toluene-ethanol-acetic acid, 65/30/5; R _f =0.48

EXAMPLE 16: Preparation of N-palmitoylethanolamine glycerophosphate

Glycerophosphorylethanolamine (2.15 g; 10 mmol) was suspended under
 15 continuous stirring at 0°C in a mixture consisting of NaOH 0.5M (40
 ml) and chloroform (20 ml). The mixture was added dropwise, over a
 period of 30 min. with palmitoyl chloride (2.75 g; 10 mmol). The
 resulting mixture was allowed to stir overnight at 0°C and then
 acidified with HCl 6. The organic phase was separated. The aqueous
 20 phase was extracted twice with chloroform (10 ml) and discarded. The
 organic extracts were washed twice with water (10 ml), combined and
 evaporated to dryness. The crude product was purified by silica gel
 column chromatography, eluting with chloroform-methanol-water,
 70/30/3.5. The fractions containing the pure product were combined.
 25 evaporated to dryness and the residue was dried in high vacuo.

The reaction yield was 86% approx.

The physico-chemical properties of N-palmitoylethanolamine

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glycerophosphate were as follows:

physical state	white amorphous powder
molecular formula	C ₂₁ H ₄₄ NO ₇ P
molecular weight	453.55
5 elemental analysis	C=55.61%; H=9.78%; N=3.09%; O=24.69%; P=6.83%
solubility in organic	
solvents	>10 mg/ml in DMSO;
solubility in water	scarcely soluble (>1 mg/ml in phosphate
10	buffer 50mM, pH 7.4
melting point	-
TLC	eluent: chloroform-methanol-water- NH ₃ (28%). 50/40/7/3; Rf=0.85

EXAMPLE 17: Preparation of N,N'-bis(4-hydroxybutyl)-trans-2-
15 dodecendiamide

Traumatic acid (2.28 g; 10 mmol) was solubilized in anhydrous dimethylformamide (40 ml) at 0°C with pyridine (2 ml) and added with N-hydroxysuccinimide (2.53 g; 22 mmol) and dicyclohexyl-carbodiimide (4.12 g; 20 mmol). The resulting mixture was stirred at 0°C for 2 hrs. 20 The urea formed was separated by filtration and discarded. The solution containing succinimide ester was stirred again at 0°C and added with 4-amino-1-butanol (2.67 g; 30 mmol). The resulting mixture was allowed to stir overnight at room temperature, then evaporated to dryness under vacuum. The residue was crystallized twice from water (60 ml). The crystalline fraction was separated by filtration, washed twice with water (5 ml) and finally dried in high vacuo. 25 The reaction yield was 92% approx.

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The physico-chemical properties of N,N'-bis-(4-hydroxybutyl)-trans-2-dodecendiamide were as follows:

physical state	white crystalline powder
molecular formula	C ₂₀ H ₃₈ N ₂ O ₄
5 molecular weight	370.53
elemental analysis	C=64.83%; H=10.34%; N=7.56%; O=17.27%
solubility in organic	
solvents	>5 mg/ml in DMSO;
10 solubility in water	scarcely soluble
melting point	150-152°C
TLC	eluent: chloroform-methanol-water- NH ₃ (28%), 80/25/2/1; Rf=0.60

EXAMPLE 18: Preparation of N-(4-hydroxybutyl)-lauroylamide

15 4-amino-1-butanol (0.89 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in ethyl acetate (50 ml) at -5°C. A solution of lauroyl chloride (2.19 g; 10 mmol) in anhydrous ethyl acetate (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C overnight, washed twice with HCl 1N (20 ml) and twice with water (15 ml). The aqueous phases were extracted twice with ethyl acetate (15 ml). The organic phases were combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from tert-butyl-methyl-ether (30 ml); the product was separated by filtration, washed twice with cold tert-butyl-methyl-ether (5 ml) and dried in high vacuo.

The reaction yield was 92% approx.

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The physico-chemical properties of N-(4-hydroxybutyl)-lauroylamide were as follows:

physical state	white crystalline powder
molecular formula	C ₁₆ H ₃₃ NO ₂
5 molecular weight	271.45
elemental analysis	C=70.80%; H=12.25%; N=5.16%; O=11.79%
solubility in organic	
solvents	>10 mg/ml in DMSO;
10	>10 mg/ml in ethanol
solubility in water	scarcely soluble
melting point	93-95°C
TLC	eluent: toluene-ethanol-acetic acid. 65/30/5; Rf=0.60

15 **EXAMPLE 19: Preparation of N-(2-hydroxyethyl)-palmitoleylamide**

A mixture of palmitoleic acid (2.54 g; 10 mmol) and triethylamine (1.06 g; 10.5 mmol) in anhydrous chloroform (100 ml), allowed to stir at -10°C in a nitrogen environment, was added dropwise, over a period of 30 min, with a solution of isobutylchloroformate (1.44 g; 10.5 mmol) in anhydrous chloroform (50 ml). The resulting mixture was stirred at -10°C for 1 hr and at 0°C for an additional 1 hr. To the mixture was added dropwise ethanolamine (0.9 g) over a period of 10 min. After an additional 2-hr stirring at 0°C, the mixture was evaporated to dryness. The residue was purified by silica gel column chromatography, eluting with chloroform-methanol, 98/2 v/v. The eluate fractions containing the product were combined and evaporated to dryness. The residue was crystallized from acetonitrile (30 ml).

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separated by filtration and dried in high vacuo.

The reaction yield was 92% approx.

The physico-chemical properties of N-(2-hydroxyethyl)-palmitoleylamide were as follows:

5	physical state	white crystalline powder
	molecular formula	C ₁₈ H ₃₅ NO ₂
	molecular weight	297.48
	elemental analysis	C=72.68%; H=11.86%; N=4.71%; O=10.76%
10	solubility in organic	
	solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol
	solubility in water	scarcely soluble
	melting point	55-57°C
15	TLC	eluent: chloroform/methanol/water- NH ₃ (28%), 80/25/2/1; Rf=0.79

EXAMPLE 20: Preparation of N-(4-hydroxy-3-methoxybenzyl)-oleoylamine

Oleic acid (2.83 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in dimethylformamide (30 ml) at 0°C and added with 20 isobutylchloroformate (1.44 g; 10.5 mmol). The resulting solution was stirred at 0°C for 20 min, then added with 4-hydroxy-3-methoxybenzylamine hydrochloride (1.90 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) and allowed to stir overnight at 0°C. The resulting mixture was added with water (90 ml) and extracted three times with 25 ethyl acetate (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), combined, decolourized with bone charcoal, dehydrated with anhydrous sodium sulphate and

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evaporated under vacuum. The residue was purified by silica gel column preparative chromatography, eluting with hexane-ethylacetate-acetic acid, 70/30/0.5. The eluate fractions containing the pure product were combined, evaporated to dryness and the residue was finally dried in 5 high vacuo.

The reaction yield was 90% approx.

The physico-chemical properties of N-(4-hydroxy-3-methoxybenzyl)-oleoylamide were as follows:

	physical state	whitish amorphous powder
10	molecular formula	C ₂₆ H ₄₃ NO ₃
	molecular weight	417.64
	elemental analysis	C=74.78%; H=10.38%; N=3.35%; O=11.49%
	solubility in organic	
15	solvents	>10 mg/ml in DMSO >10 mg/ml in ethanol
	solubility in water	scarcely soluble
	melting point	-
	TLC	eluent: toluene-ethanol-acetic acid. 65/30/5; Rf=0.66
20		

EXAMPLE 21: Preparation of N-(4-hydroxy-3-methoxybenzyl)-palmitoylamide

4-hydroxy-3-methoxybenzylamide hydrochloride (1.90 g; 10 mmol) and triethylamine (2.26 g; 22 mmol) were solubilized in dimethylformamide 25 (30 ml) at 0°C. A solution of palmitoyl chloride (2.75 g; 10 mmol) in dimethylformamide (15 ml) was added dropwise, over a period of 30 min, under continuous stirring. The resulting mixture was stirred at 0°C

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overnight, then added with water (90 ml) and extracted three times with tert-butyl-methyl-ether (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), then combined, decolourized with bone charcoal, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from tert-butyl-methyl-ether (30 ml). The product was separated by filtration, washed twice with cold tert-butylmethyl ether (5 ml) and finally dried in high vacuo.

The reaction yield was 89% approx.

10 The physico-chemical properties of N-(4-hydroxy-3-methoxybenzyl)-palmitoylamide were as follows:

physical state white crystalline powder

molecular formula C₂₄H₄₁NO₃

molecular weight 391.60

15 elemental analysis C=73.61%; H=10.55%; N=3.58%;
O=12.26%

solubility in organic

solvents >10 mg/ml in DMSO

>10 mg/ml in ethanol

20 solubility in water scarcely soluble

melting point -

TLC eluent: toluene-ethanol-acetic acid.
65/30/5; Rf=0.65

EXAMPLE 22: Preparation of N-(2-hydroxyethyl)-lauroylamide

25 A mixture of lauric acid (2.00 g; 10 mmol) and ethanolamine (0.916 g; 15 mmol) was fed to a flask provided with reflux condenser and heated on an oil bath to 160°C for 6 hrs.

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The reaction mixture was directly crystallized from ethanol 80% (50 ml). The crystalline reaction was separated by filtration, washed three times with cold ethanol 80% (10 ml) and finally dried in high vacuo.

5 The reaction yield was 90% approx.

The physico-chemical properties of N-(2-hydroxyethyl)-lauroylamide were as follows:

physical state white crystalline powder

molecular formula C₁₄H₂₉NO₂

10 molecular weight 243.39

elemental analysis C=69.09%; H=12.01%; N=5.76%;
O=13.15%

solubility in organic

solvents >10 mg/ml in DMSO

15 >10 mg/ml in chloroform

solubility in water scarcely soluble

melting point 85-87°C

TLC eluent: chloroform-methanol-water-
NH₃ (28%). 80/25/2/1; R_f=0.83

20 EXAMPLE 23: Preparation of N-(2-hydroxyethyl)-stearoylamide

A mixture of stearic acid (2.85 g; 10 mmol) and ethanolamine (0.916 g; 15 mmol) was fed to a flask provided with reflux condenser and heated on an oil bath to 160°C for 6 hrs.

The reaction mixture was directly crystallized from ethanol 95% (50 ml). The crystalline fraction was separated by filtration, washed three times with cold ethanol 95% (10 ml) and finally dried in high vacuo.

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The reaction yield was 90% approx.

The physico-chemical properties of N-(2-hydroxyethyl)-stearoyl amide were as follows:

	physical state	white crystalline powder
5	molecular formula	C ₂₀ H ₄₁ NO ₂
	molecular weight	327.55
	elemental analysis	C=73.34%; H=12.62%; N=4.28%; O=9.77%
	solubility in organic	
10	solvents	>5 mg/ml in chloroform
	solubility in water	scarcely soluble
	melting point	98-100°C
	TLC	eluent: chloroform-methanol-water- NH ₃ (28%), 80/25/2/1; R _f =0.87

15 EXAMPLE 24: Preparation of N-(3-hydroxypropyl)-palmitamide

A mixture of palmitic acid (2.75 g; 10 mmol) and propanolamine (1.13 g; 15 mmol) was fed to a flask provided with reflux condenser and heated on an oil bath to 160°C for 6 hrs.

20 The reaction mixture was directly crystallized from ethanol 95% (50 ml). The crystalline fraction was separated by filtration, washed three times with cold ethanol 95% (10 ml) and finally dried in high vacuo.

The reaction yield was 80% approx.

25 The physico-chemical properties of N-(3-hydroxypropyl)-palmitamide were as follows:

physical state	white crystalline powder
molecular formula	C ₁₉ H ₃₉ NO ₂

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	molecular weight	313.53
	elemental analysis	C=72.79%; H=12.54%; N=4.47%; O=10.21%
	solubility in organic	
5	solvents	> 3 mg/ml in DMSO >10 mg/ml in n-octanol
	solubility in water	scarcely soluble
	melting point	91-93°C
	TLC	eluent: chloroform-methanol. 95/5; Rf=0.40
10		

EXAMPLE 25: Preparation of N-palmitoylethanolamide

According to the method described by E.T. Roe et al., (J. Am. Chem. Soc., 74, 3442-3443, 1952), the synthesis of N-palmitoylethanolamide was obtained by refluxing ethanolamine with palmitic acid. In 15 particular, palmitic acid (1 mol) was caused to react with ethanolamine (1.5 mol) in ethyl ether for 5-6 hrs under nitrogen atmosphere.

The reaction product was extracted from the reaction mixture and crystallized from ethanol 95% at 0°C. The melting point of N-PEA found 20 was 94-95°C.

The physico-chemical properties of N-palmitoylethanolamide were as follows:

	physical state	crystalline powder
	molecular formula	C ₁₈ H ₃₇ NO ₂
25	molecular weight	299.48
	elemental analysis	C=72.19%; H=12.45%; N=4.68%; O=10.69%

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solubility in organic

solvents in hot MeOH, CHCl_3 , DMSO

solubility in water insoluble

melting point 94-95°C

5 TLC eluent: toluene-chloroform, 9/1;
Rf=0.75

EXAMPLE 26: Preparation of N,N'-bis(2-hydroxyethyl)-trans-2-dodecenediamide

A mixture of traumatic acid (4.57 g; 20 mmol) and triethylamine (4.26 g; 42 mmol) in anhydrous THF (150 ml) allowed to stir at -10°C, was dropwise added, over a period of 30 min, with a solution of isobutylchloroformate (5.74 g; 42 mmol) in THF (50 ml). The mixture was stirred at -10°C for 2 hrs and at 0°C for additional 15 hrs and added dropwise, over a period of 30 min, with ethanolamine (3.5 g).

10 After stirring for additional 6 hrs at 0°C, the resulting suspension was filtered. The filtrate was discarded and the solid was dried under vacuum. The crude product obtained was crystallized from water (100 ml). The crystalline fraction was separated by filtration, washed three times with water (20 ml) and finally dried in high vacuo.

15 The reaction yield was 78% approx.

20 The physico-chemical properties of N,N'-bis(2-hydroxyethyl)-trans-2-dodecenediamide were as follows:

physical state white crystalline powder

molecular formula $\text{C}_{16}\text{H}_{30}\text{N}_2\text{O}_4$

molecular weight 314.43

25 elemental analysis C=61.12%; H=9.62%; N=8.91%;
O=20.35%

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solubility in organic	
solvents	>10 mg/ml in DMSO
	>10 mg/ml in ethanol
solubility in water	
	>10 mg/ml at 95°C
5 melting point	134-136°C
TLC	eluent: chloroform-methanol-water-
	NH ₃ (28%), 80/25/2/1; Rf=0.57

EXAMPLE 27: Preparation of N-palmitoylethanolamide phosphate

N-(2-hydroxyethyl)-palmitoylethanolamide (3.0 g; 10 mmol) was solubilized in anhydrous methanesulphonic acid (10 ml) under stirring 10 at 0°C and added with phosphoric anhydride (2.12 g; 15 mmol). The resulting mixture was stirred at 0°C for 25 hrs. The reaction mixture was added with ether to complete product precipitation. The precipitate was separated by centrifugation, dried under vacuum, washed with cold water and dried again under vacuum. The crude product obtained was hot washed with tert-butyl-methyl-ether (50 ml) and then 15 crystallized from isopropanol (50 ml). The crystalline fraction was separated by filtration, washed three times with cold isopropanol (10 ml) and finally dried in high vacuo.

The reaction yield was 83% approx.

20 The physico-chemical properties of N-palmitoylethanolamide phosphate were as follows:

physical state	white crystalline powder
molecular formula	C ₁₈ H ₃₈ NO ₅ P
molecular weight	379.48
25 elemental analysis	C=56.97%; H=10.09%; N=3.69%; O=21.08%; P=8.16%

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solubility in organic

solvents >10 mg/ml in DMSO

solubility in water scarcely soluble (>1 mg/ml in phosphate buffer (50 mM), pH 7.4, NaCl 0.9%

5 melting point -

TLC eluent: chloroform-methanol-water-
NH₃ (30%), 50/40/7/3; Rf=0.38

EXAMPLE 28: Preparation of N-(2-hydroxyethyl)-10-undecenoylamide

10-Undecenoic acid (1.84 g; 10 mmol) was solubilized in anhydrous
10 methanol (30 ml) and added with anhydrous H⁺ sulphonic resin Dowex
50X8^(R). The mixture was stirred at 30°C for 72 hrs. The resin was
separated by filtration. the solution was evaporated to dryness and
the residue was added with ethanolamine (0.916 g) and allowed to stir
15 at 60°C for 20 hrs. The reaction mixture was directly crystallized
from methanol-water 2/1 (50 ml) and the crystalline fraction was
separated by filtration, washed three times with cold water (10 ml)
and finally dried in high vacuo.

The reaction yield was 70% approx.

20 The physico-chemical properties of N-(2-hydroxyethyl)-10-undecenoylamide were as follows:

physical state white crystalline powder

molecular formula C₁₃H₂₅NO₂

molecular weight 227.35

elemental analysis C=68.68%; H=11.08%; N=6.16%;

25 O=14.07%

solubility in organic

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	solvents	>10 mg/ml in DMSO
		>10 mg/ml in ethanol
	solubility in water	scarcely soluble
	melting point	68.5-70.5°C
5	TLC	eluent: chloroform-methanol-water- NH ₃ (28%), 88/25/2/1; Rf=0.57

EXAMPLE 29: Preparation of N-palmitoyl-morpholine

Morpholine (0.87 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous dimethylformamide (50 ml) at 0°C. A solution 10 of palmitoylchloride (2.74 g; 10 mmol) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was evaporated to dryness under vacuum. The crude residue was washed in suspension with water, 15 crystallized from cold ethanol 70% (30 ml). The product was separated by filtration, washed twice with cold ethanol 70% (5 ml) and dried in high vacuo.

The reaction yield was 92% approx.

The physico-chemical properties of N-palmitoylmorpholine were as 20 follows:

physical state	white crystalline powder
molecular formula	C ₂₀ H ₃₉ NO ₂
molecular weight	325.53
elemental analysis	C=73.79%; H=12.08%; N=4.30%;
25	O=9.83%
solubility in organic	
solvents	>10 mg/ml in DMSO

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	>10 mg/ml in hot ethanol
solubility in water	scarcely soluble
melting point	45-46°C
TLC	eluent: chloroform-methanol, 95/5; Rf=0.94

5

EXAMPLE 30: Preparation of N-palmitoyl-[R(-)-2-amino-1-propanol]

R(-)-2-amino-1-propanol (0.75 g; 10 mmol) and triethylamine (1.1 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (30 ml) at 0°C. A solution of palmitoyl chloride (2.75 g; 10 mmol) in anhydrous tetrahydrofuran (15 ml) was slowly added over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was kept under stirring overnight at 0°C, added with water (90 ml) and extracted three times with ethyle acetate (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml). combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from methanol (30 ml). The product was separated by filtration, washed twice with cold methanol (5 ml) and dried in high vacuo.

15

The reaction yield was 89% approx.

20

The physico-chemical properties of N-palmitoyl-[R(-)-2-amino-1-propanol] were as follows:

25

physical state	white crystalline powder
molecular formula	C ₁₉ H ₃₉ NO ₂
molecular weight	313.52
elemental analysis	C=72.79%; H=12.54%; N=4.47%; O=10.21%
solubility in organic solvents	>10 mg/ml in ethanol;

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	>5 mg/ml in DMSO;
solubility in water	scarcely soluble
melting point	87-89°C
TLC	eluent: toluene-ethanol-acetic acid.
5	65/30/5; Rf=0.63

EXAMPLE 31: Preparation of N-palmitoyl-[S(+)-2-amino-1-propanol]

S(+)-2-amino-1-propanol (0.75 g; 10 mmol) and triethylamine (1.1 g; 11 mmol) were solubilized in chloroform (30 ml) at 0°C. A solution of palmitoyl chloride (2.75 g; 10 mmol) in chloroform (15 ml) was slowly added dropwise over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was kept under stirring overnight at 0°C and added with water (60 ml); the organic phases were separated and the aqueous phases were extracted twice with chloroform (25 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from methanol (30 ml). The product was separated by filtration, washed twice with cold methanol (5 ml) and dried in high vacuo.

The reaction yield was 93% approx.

20 The physico-chemical properties of N-palmitoyl-[S(+)-2-amino-1-propanol] were as follows:

physical state white crystalline powder

molecular formula C₁₉H₃₉NO₂

molecular weight 313.52

25 elemental analysis C=72.79%; H=12.54%; N=4.47%; O=10.21%

solubility in organic

solvents >10 mg/ml in ethanol;

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>5 mg/ml in DMSO;
solubility in water scarcely soluble
melting point 87-89°C
TLC eluent: toluene-ethanol-acetic acid.
5 65/30/5; Rf=0.63

EXAMPLE 32: Preparation of N-palmitoyl-[R(-)-1-amino-2- propanol]

R(-)-1-amino-2-propanol (0.75 g; 10 mmol) and triethylamine (1.1 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (30 ml) at 0°C. A solution of palmitoyl chloride (2.75 g; 10 mmol) in anhydrous tetrahydrofuran (15 ml) was slowly added dropwise over a period of 30 min. under continuous stirring at 0°C. The resulting mixture was kept under stirring overnight at 0°C, added with water (90 ml) and extracted three times with ethyle acetate (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml). 10 combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from methanol (30 ml). The product was separated by filtration. washed twice with cold methanol (5 ml) and dried in high vacuo.

The reaction yield was 92% approx.

20 The physico-chemical properties of N-palmitoyl-[R(-)-1-amino-2- propanol] were as follows:

physical state white crystalline powder

molecular formula C₁₉H₃₉NO₂

molecular weight 313.52

25 elemental analysis C=72.79%; H=12.54%; N=4.47%; O=10.21%

solubility in organic

solvents >5 mg/ml in ethanol;

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solubility in water	scarcely soluble
melting point	88-90°C
TLC	eluent: toluene-ethanol-acetic acid. 65/30/5; Rf=0.63

5 **EXAMPLE 33: Preparation of N-palmitoyl-[S(+)-1-amino-2-propanol]**

S(+)-1-amino-2-propanol (0.75 g; 10 mmol) and triethylamine (1.1 g; 11 mmol) were solubilized in chloroform (30 ml) at 0°C. A solution of palmitoyl chloride (2.75 g; 10 mmol) in chloroform (15 ml) was slowly added dropwise over a period of 30 min, under continuous stirring at 10 0°C. The resulting mixture was kept under stirring overnight at 0°C and added with water (60 ml); the organic phases were separated and the aqueous phases were extracted twice with chloroform (25 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and 15 evaporated under vacuum. The residue was crystallized from methanol (30 ml). The product was separated by filtration, washed twice with cold methanol (5 ml) and dried in high vacuo.

The reaction yield was 88% approx.

20 The physico-chemical properties of N-palmitoyl-[S(+)-1-amino-2-propanol] were as follows:

physical state	white crystalline powder
molecular formula	C ₁₉ H ₃₉ NO ₂
molecular weight	313.52
elemental analysis	C=72.79%; H=12.54%; N=4.47%; O=10.21%
25 solubility in organic solvents	>5 mg/ml in ethanol
solubility in water	scarcely soluble

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melting point	87-89°C
TLC	eluent: toluene-ethanol-acetic acid, 65/30/5; Rf=0.63

EXAMPLE 34: Preparation of N-(p-hydroxy-phenylethyl)-palmitoyl amide

2-(4-hydroxy-phenyl)-ethylamine (1.37 g; 10 mmol) and triethylamine

5 (1.13 g; 11 mmol) were solubilized in tetrahydrofuran (30 ml) at 0°C.

A solution of palmitoyl chloride (2.75 g; 10 mmol) in tetrahydrofuran

(15 ml) was added dropwise, over a period of 30 min, under continuous

stirring at 0°C. The resulting mixture was stirred overnight at 0°C.

added with water (90 ml) and extracted three times with tert-butyl-

10 methylether. The organic phases were washed twice with HCl 1N (20 ml)

and twice with water (15 ml), combined, decolorated with animal

carbon, dehydrated with anhydrous sodium sulphate and evaporated under

vacuum. The residue was crystallized from tert-butyl-methylether (30

ml). The product was separated by filtration, washed twice with cold

15 tert-butyl-methylether (5 ml) and dried in high vacuo.

The reaction yield was 88% approx.

The physico-chemical properties of N-(p-hydroxy-phenylethyl)-palmitoyl amide were as follows:

physical state	white crystalline powder
----------------	--------------------------

20 molecular formula C₂₄H₄₁NO₂

molecular weight 375.60

elemental analysis C=76.75%; H=11.00%; N=3.73%; O=8.52%

solubility in organic

solvents >10 mg/ml in DMSO;

25 >10 mg/ml in ethanol;

solubility in water scarcely soluble

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melting point	105-106°C
TLC	eluent: toluene-ethanol-acetic acid, 65:30:5; Rf = 0.64

EXAMPLE 35: Preparation of N-(2-methoxy-ethyl)-palmitoylamide

5 2-methoxy-ethylamine (0.9 g; 12 mmol) and triethylamine (1.52 g; 15 mmol) were solubilized in tetrahydrofuran (30 ml) at 0°C. A solution of palmitoyl chloride (2.75 g; 10 mmol) in tetrahydrofuran (15 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred overnight at 0°C, added with 10 water (90 ml) and extracted three times with ethyle acetate (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from tert-butyl-methylether (30 ml). The product was separated by 15 filtration, washed twice with cold tert-butyl-methylether (5 ml) and dried in high vacuo.

The reaction yield was 93% approx.

The physico-chemical properties of N-(2-methoxy-ethyl)-palmitoylamide were as follows:

20 physical state	white crystalline powder
molecular formula	C ₁₉ H ₃₉ NO ₂
molecular weight	313.52
elemental analysis	C=72.79%; H=12.54%; N=4.47%; O=10.21%
solubility in organic	
25 solvents	>10 mg/ml in ethanol
solubility in water	scarcely soluble
melting point	73-75°C

EXAMPLE 36: Preparation of N-lauroyl-morpholine

Morpholine (0.87 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous tetrahydrofuran (50 ml) at 0°C. A solution of lauroyl chloride (2.19 g; 10 mmol) in anhydrous tetrahydrofuran (20 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 5 hrs. The resulting suspension was added with water (90 ml) and extracted three times with tert-butyl-methylether (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), combined, decolorized with animal charcoal, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was purified by silica gel column chromatography eluting with a mixture of hexane/ethyle acetate/acetic acid. 80/20/0.5. The fractions containing the pure product were combined and evaporated to dryness. The residue was dried in high vacuum.

The reaction yield was 90% approx.

20 The physico-chemical properties of N-lauroyl-morpholine were as follows:

physical state deliquescent amorphous powder

molecular formula $C_{16}H_{31}NO_2$

molecular weight 269.43

25 elemental analysis C=71.33%; H=11.60%; N=5.20%; O=11.88%

solubility in organic

solvents >10 mg/ml in DMSO;

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	>10 mg/ml in ethanol;
solubility in water	scarcely soluble
melting point	21°C
TLC	eluent: hexane-ethyl acetate-acetic acid.
5	75/24/1; Rf=0.25

EXAMPLE 37: Preparation of N-stearoyl-morpholine

Morpholine (0.87 g; 10 mmol) and triethylamine (1.13 g; 11 mmol) were solubilized in anhydrous dimethylformamide (50 ml) at 0°C. A solution of stearoyl chloride (3.02 g; 10 mmol) in anhydrous dimethylformamide (20 ml) was added dropwise, over a period of 30 min. under continuous stirring at 0°C. The resulting mixture was stirred at 0°C for 1 hr and at room temperature for additional 20 hrs. The resulting suspension was evaporated to dryness under vacuo. The crude product was washed in suspension with water, crystallized first from cold ethanol (30 ml) and then from tert-butyl-methylether (30 ml). The product was separated by filtration, washed twice with cold tert-butyl-methylether (5 ml) and dried in high vacuo.

The reaction yield was 89% approx.

The physico-chemical properties of N-stearoyl-morpholine were as follows:

20	physical state	white crystalline powder
molecular formula	$C_{22}H_{43}NO_2$	
molecular weight	325.59	
elemental analysis	C=74.73%; H=12.26%; N=3.96%; O=9.05%	
solubility in organic		
25	solvents	>10 mg/ml in hot ethanol
solubility in water	scarcely soluble	
melting point	60-61°C	

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TLC eluent: toluene-ethanol-acetic acid

65/30/5; Rf=0.67

EXAMPLE 38: Preparation of 1,10-bis-morpholinylcarbonyl-*trans*-1-decene

Traumatic acid (2.28 g; 10 mmol) was solubilized in anhydrous

5 dimethylformamide (40 ml) at 0°C with pyridine (2 ml). N-
 hydroxysuccinimide (2.53 g. 22 mmol) and dicyclohexylcarbodiimide
 (4.12 g. 20 mmol) were added and the resulting mixture was stirred at
 0°C for 2 hours. The urea so formed was separated by filtration and
 discarded, while the solution containing the succinimidic ester was
 10 further stirred at 0°C and added with morpholine (2.61 g. 30 mmol).

The resulting mixture was stirred overnight at 30°C and evaporated to dryness under vacuum. The crude residue was taken up with water (40 ml) and extracted three times with ethyl acetate (40 ml). The organic phases were combined, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized twice from tert-butyl-methylether (50 ml). The product was separated by filtration, washed twice with cold tert-butyl-methylether (5 ml) and dried in high vacuo.

The reaction yield was 89% approx.

20 The physico-chemical properties of 1,10-bis-morpholinylcarbonyl-trans-
1 decene were as follows:

physical state white crystalline powder

molecular formula C₂₀H₃₄N₂O₁₁

molecular weight 366.51

25 elemental analysis C=65.54%; H=9.35%; N=7.64%; O=17.46%

solubility in organic

solvents >10 mg/ml in DMSO;

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	>10 mg/ml in ethanol;
solubility in water	scarcely soluble
melting point	85-87°C
TLC	eluent: toluene-ethanol-acetic acid.
5	65/30/5; Rf=0.55

EXAMPLE 39: Preparation of N-(4-hydroxy-3-methoxybenzyl)-stearoylamide

4-hydroxy-3-methoxybenzylamine hydrochloride (1.90 g, 10 mmol) and triethylamine (2.26 g; 22 mmol) were solubilized in dimethylformamide (30 ml) at 0°C. A solution of steryl chloride (3.03 g; 10 mmol) in dimethylformamide (15 ml) was added dropwise, over a period of 30 min, under continuous stirring at 0°C. The resulting mixture was stirred overnight at 0°C; then the mixture was added with water (90 ml) and extracted three times with tert-butyl-methylether (40 ml). The organic phases were washed twice with HCl 1N (20 ml) and twice with water (15 ml), combined, decolorized with animal charcoal, dehydrated with anhydrous sodium sulphate and evaporated under vacuum. The residue was crystallized from tert-butyl-methylether (30 ml). The product was separated by filtration, washed twice with cold tert-butyl-methylether (5 ml) and dried in high vacuo.

The reaction yield was 91% approx.

The physico-chemical properties of N-(4-hydroxy-3-methoxybenzyl)-stearoylamide were as follows:

physical state	white crystalline powder
25 molecular formula	C ₂₆ H ₄₅ NO ₃
molecular weight	419.65
elemental analysis	C=74.42%; H=10.81%; N=3.34%; O=11.44%
solubility in organic	

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solvents	>10 mg/ml in DMSO; >10 mg/ml in ethanol;
solubility in water	scarcely soluble
melting point	87-89°C
5 TLC	eluent: toluene-ethanol-acetic acid. 65/30/5; Rf=0.66

As already mentioned, the amides of mono and bicarboxylic acids with aminoalcohols and aminoethers according to the present invention, corresponding to formula (I) reported hereinabove, can selectively bind to CB2 peripheral receptor, acting as competitive agonists and displacing from said receptor the natural and synthetic cannabinoids already known. Furthermore, said amides are capable of functionally activating CB2 receptor, by mimicking the non-psychoactive biological effects of cannabinoids and acting with a potency higher than or comparable with the one of cannabinoids.

The Applicant has also surprisingly found that CB2 cannabinoid peripheral receptor is present on mast cell. Furthermore, the Applicant has unexpectedly found that CB2 cannabinoid peripheral receptor is functionally expressed also on non-immune cells or tissue; in particular, the Applicant has found that CB2 is functionally expressed in Nervous System on cerebellar granule cells, indicating that such receptor can modulate also non-immune cell functions. This extremely important aspect of the present invention will be illustrated in detail hereinbelow.

25 A) Identification of CB2 receptor on mast cells and non-immune cells.

In order to verify that CB2 cannabinoid peripheral receptor is present on mast cells and on non-immune cells (i.e. neurons), the presence of

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messenger RNA for said receptor was investigated in primary cultures of rat peritoneal mast cells, in a basophilic-mastocyte cell line from rat solid tumour (Rat Basophilic Leukemia, RBL-2H3) and in mouse cerebellar granule cell culture, in comparison with rat spleen homogenate tissues, where the presence of said receptor is acknowledged.

Both Polymerase Chain Reaction and in situ hybridisation technique were used.

i) Cannabinoid Peripheral Receptor specific hybridisation amplified by Polymerase Chain Reaction (PCR).

Extraction of total mRNA from tissues and cells

Total RNA was extracted from rat peritoneal mast cells, RBL-2H3 cells and rat spleen, according to the method described by Chumczynski and Sacchi (Anal. Biochem., 162, 156-159, 1987).

5 5×10^6 mast cells, cells RBL-2H3, or 50 mg of spleen were separately homogenized in a 1.5 ml polypropylene tube Eppendorf [®] (safe-lock type) with guanidinium isothiocyanate 5M (600 μ l). Each tube was then supplied with sodium acetate 2M (60 μ l), pH 5, and phenol (600 μ l) saturated in buffer Tris-HCl 1M, pH 7.0. The samples were briefly stirred by vortex mixing (Heidolph, type REAX 2000 [®]), and then added with chloroform-isoamyl alcohol (49:1 v/v) (120 μ l). After agitation for 15 sec. by vortex mixing and incubation in ice for 15 min. the samples were centrifuged at 15.000xg at 4°C for 15 min. 600 μ l of the upper aqueous phase was withdrawn and placed in another tube, having care to avoid any contact with the interface of the organic lower phase, which was discarded. The aqueous phase was added with isopropanol (600 μ l) and, after agitation, the samples were incubated

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at -20°C for at least 60 min and centrifuged at 15,000 xg at 4°C for 5 min. The supernatant was discarded. The pellet was washed with ethanol 80% and then with absolute ethanol, concentrated to dryness and suspended again in water (20 µl). 2 µl of the sample were used for the 5 spectrophotometric determination of RNA concentration at the wavelength of 260 nm and for the visualization with ethidium bromide on agarose gel at 1%.

Reverse transcription and amplification by Polymerase Chain Reaction (PCR) of CB2 receptor

10 1 µg of total RNA withdrawn from each of the above samples was subjected to reverse transcription, as described by Leon et al. (PNAS. 91:3739-3743, 1994), using the reverse transcriptase of murine moloney leukaemic virus and 50 pmol of a synthetic oligonucleotide SEQ ID NO :1 of sequence: 5'-TAGGTAGGAGATCAAGCG-3', complementary and 15 antiparallel to the mRNA coding for cannabinoid peripheral receptor (Munro et al.. Nature 365, 61- 65, 1993), in a total volume of 20 µl. After 1-hr reaction at 37°C, the volume of the transcription product was brought to 100 µl with water and one fourth of the final volume was subjected to amplification by means of PCR. Said operation was 20 conducted in 500 µl thin-walled test tubes, filled up to a final volume of 100 µl with a solution having the following composition: 50 mM TRIS HCl, pH 8.3, at 25°C: 75 mM KCl; 2.5 mM MgCl₂; 25 10 mM DTT (dithiothreitol); 0.2 mM dATP, dCTP, dGTP and dTTP; 50 pmol sense primer SEQ ID NO:2: (5'-TTTCACGGTGTGGACTCC-3');

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50 pmol antisense primer SEQ ID NO:3: (5'-TAGGTAGGAGATCAAGCG-3');
0.551 *Taq* Pol (*Taq* polymerase) 2.5 U/ μ l (Polymerase Stoffel fragment,
Cetus/Perkin-Elmer[®]);
3% formamide.

5 Primers were specific for cannabinoid peripheral receptor. PCR was
carried out for 35 thermal cycles in apparatus mod. 9600 of
Cetus/Perkin Elmer[®], according to the following procedure:

	Temperature	Duration
9	95°C	1 min
10	54°C	1 min
	72°C	1 min

Once the reaction had been completed, 30 μ l of the reaction volume was
electrophoresed on 1% agarose gel in order to visualize the
amplification products, then transferred and immobilized on a nylon
15 filter for inner-probe hybridization to the two PCR primers.

Specific hybridization with inner oligonucleotide

The identity of the amplification product was determined by
hybridization with a synthetic oligonucleotide SEQ ID NO:4,
complementary to amplified sequences of cannabinoid peripheral
20 receptor, labelled with radioactive tracers.

The sequence of SEQ ID NO:4 was as follows:

5'-GGTGACGAGAGCTTGTAGGTAGGTGGTAGCACAGACATAGGTA-3'

Radioactive labelling was carried out at 37°C for 1 hr, using 5 pmol
inner oligonucleotide. as follows:

25 33 pmol (α ³²P)-dATP (New England Nuclear);

20 U Terminal Deoxynucleotide Transferase (US Biochemical);

100 mM sodium cacodylate (pH 7.2)

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2 mM CoCl₂

0.2 mM 2-mercaptoethanol

in a final volume of 20 µl.

The labelled products were purified by chromatography on Sephadex G-50[®] column, and added to the hybridization solution containing the filter with the PCR-amplified products. Hybridization and washings were carried out under standard conditions, as described by Sambrook, Fritsch and Maniatis (Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory Press. 1989).

10 The hybridized filter was exposed to a autoradiographic film for the detection of the amplified bands of the cannabinoid peripheral receptor messenger.

ii) mRNA - Peripheral Cannabinoid Receptor *in situ* hybridisation.

The brains of adult male mice (20-22 g) were frozen in 2-methyl-butane at -80°C. sectioned with a cryostat and the 12mm coronal sections were thaw-mounted on poly-L-lysine coated slides. All sections were then fixed in 4% paraformaldehyde (PFA) and dehydrated in a graded series of ethanol, including a 5 min. incubation in chloroform and air dried. Mouse cerebellar granule cells (15 day *in vitro*-DIV) cultured on poly-L-lysine coated coverslips were fixed in 4% PFA, washed twice in PBS and permeabilized in 70% ethanol at 4°C for 48 hrs, dehydrated in higher graded ethanol and air dried. To detect the CB2-specific mRNA, was chosen the synthetic oligonucleotide (45 mers) CB2 (SEQ ID NO:4: 5'-GGTGACGAGAGCTTGTAGGTAGGTGGGTACCAACAGACATAGGTA-3 according to Munro et al.. 1993, cited reference). A random sequence was utilized for control sections. All oligonucleotides were tailed with ³⁵S-dATP (NEN) using terminal deoxynucleotidyl-transferase (Pharmacia) to a specific

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activity of 10^9 cpm/mg. All sections were hybridized in standard solutions (*) with 1.5 10^7 dpm/ml overnight at 42°C in a humidified chamber. Sections were then washed once in 1X SSC/0.1%(**) SDS at 55°C for 30 min., then twice in 1X SSC at 55°C for 15 min., followed by 5 0.1X SSC at 25°C for 30 min.. rinsed in autoclaved water for 2min.. dehydrated in ethanol and air dried. The slides were subsequently dipped in photoemulsion (Ilford K.5 diluted 1:1 in water), exposed for 5 weeks at 4°C, developed (Ilford Phenisol), fixed (Ilford Hypam), and counterstained with cresyl violet.

10 Standard Solution preparation

(*) Hybridisation cocktail Formamide: 5 ml. 50%; SSC 20x: 2 ml. 4 x; Denhardt's 50x: 0.2 ml; 1x Lauryl-sacarosyl 20%: 0.5 ml. 1%; Destran sulphate: 1 g. 10% : 200 mM Phosphate buffer pH 7.0: 1 ml. 20 mM. water-DEPC q.s.t o 10 ml. Filtrate using 0.45 μ m filter and store at - 15 20°C.

(**) Sodium Citrate Solution SCS 20x. Sodium chloride 175.3g and sodium citrate 88.2g were dissolved in 800ml DEPC-water; NaOH 10M was used to adjust the pH 7.2. Add DEPC-water q.s. to 1000ml for autoclave procedure.

20 The autoradiographic examination shows that rat peritoneal mast cells and the cousin RBL-2H3 cells, cerebellar granule cells and cerebellum express, similarly to spleen preparations, mRNA specific to peripheral cannabinoid receptor CB2. This finding is clearly shown in Figures 1 and 2. Figure 1 illustrates the specific hybridization of cannabinoid 25 peripheral receptor amplified by Polymerase Chain Reaction (PCR) in (A) rat spleen, (B) rat peritoneal mast cell cultures and (C) RBL-2H3 cell cultures (C). Figure 2 illustrates the specific mRNA-CB2

peripheral cannabinoid receptor *in situ* hybridisation.

B) Binding assays

Specific binding assays were conducted for the purpose of checking whether the receptor was functionally expressed and whether the amides forming the object of the present invention were able to bind to CB2 receptor in a specific and competitive manner, compared with the cannabinoids having a known affinity to the receptor and compared with anandamide, the endogenous ligand of CB1 receptor.

a) Preparation of plasmatic membranes of RBL-2H3 cells

10 RBL-2H3 cells (100×10^6), frozen at -80°C , were thawed with 4 ml of Tris-HCl buffer solution 50 mM (pH 7.4), added with 0.25% w/v of Tripsin inhibitor, type II-S: soy bean^R (distributed by Sigma). The cells were resuspended in the buffer and homogenized. The homogenate was centrifuged at 1500 xg at 4°C for 10 min. The supernatant obtained
15 was collected and the precipitate was resuspended in 4 ml of the previous buffer. The resuspended precipitate was homogenized and centrifuged again at 1500 xg at 4°C for 5-10 min. The resulting supernatant was combined to the supernatant previously obtained and centrifuged at 5000 xg for 10 min. After centrifugation, the supernatant was collected and further centrifuged at 40,000 xg at 4°C for 30 min. The resulting precipitate was resuspended in 0.5 ml of the buffer solution described above, with addition of 1% Bovine Serum
20 Albumin fatty acid free. The obtained suspension was centrifuged at 40,000 xg at 4°C for 30 min. The resulting precipitate was collected
25 and resuspended in buffer solution containing 50 mM Tris HCl, 1 mM Tris-EDTA, 3 mM MgCl₂, pH 7.4, in order to obtain a proteic concentration of 1 $\mu\text{g}/\mu\text{l}$ approx. This preparation may be used fresh, or

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frozen at -80°C and utilized within few days.

b) Binding assays conditions to the preparation of RBL-2H3 cell membranes

Saturation curve of labelled receptor agonist ^3H -WIN 55.212-2 (specific activity 44 Ci/mmol, distributed by New England Nuclear): silanized plastic test tubes were fed, in the order, with binding buffer (50 mM Tris HCl, 1 mM Tris-EDTA, 3 mM MgCl_2 , 0.5% w/v Bovine Serum Albumin fatty acid-free) to a final volume of 0.5 ml, decreasing doses of ^3H -WIN 55.212-2 from 0.5 to 20 nM (final) and 30 μg membrane proteins prepared in item a). The resulting binding mixture was incubated at 30°C for 60 min under stirring and centrifuged at 40,000 xg at 20°C for 15 min. After centrifugation, an aliquot portion of the supernatant was collected to calculate the concentration of the ligand not associated to the membranes. After removal of the supernatant residue, the precipitate was washed with 1 ml PBS (Phosphate Buffer Solution) containing 0.5% Bovine Serum Albumin, taken up with 50 μl of a mixture of ethanol and 1% Triton X-100[®] (50/50 v/v), incubated at 37°C for 20 min and resuspended.

The resuspended material was added and mixed with scintillator liquid (3 ml) and placed in a "β-counter" for 5 min. To evaluate the "Aspecific Binding", the predetermined test tubes were fed first with unlabelled receptor agonist WIN 55.212-2 at a final concentration of 15 M and then with the labelled ligand. Said binding gives $K_d = 5-10$ nM and $B_{max} = 100-250 \text{ pM}$.

25 ^3H -WIN 55.212-2 binding displacement

For these competitions, ^3H -WIN 55.212-2 was used at a concentration of 3 μM . Non-radioactive WIN 55.212-2 (1 μM) was used to inhibit specific

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binding. The competitors nabilone [3-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo-[b,d]-pyran-9-one] and anandamide were solubilized in 0.1% ethanol, whereas the products forming the object of the present invention were 5 solubilized in 0.1% DMSO. The competitors were added to the binding mixture prior to the labelled ligand. In both cases, for an evaluation of the reference specific binding, ethanol and DMSO were added at the same final concentration to the total binding and to the aspecific one, also in the absence of competitors.

10 The aforementioned binding assays provided evidence that the receptor expression was complete and functional.

The data of Tables 1 and 2 show that the amidic derivatives according to the present invention, like anandamide and the cannabinoid Nabilone, are able to bind to the receptor and to compete, with 15 different potency, with the synthetic radioligand WIN 55,212-2.

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Table 1 - Competitive inhibition of [³H] WIN 55,212-2 specific binding by the compounds of the invention. [³H] WIN 55,212-2 was used at a concentration of 3 μ M, whereas the other compounds were used at a concentration of 10 μ M.

Competitor	Specific binding	% Displacement
0.1% DMSO*	1433	-
Example 1	325	77
Example 19	-	100
Example 22	400	72
Example 25	1103	23
Example 26	402	72
Example 27	843	42
Example 28	325	77
0.1% Ethanol**	1843	-
anandamide	99	95
Nabilone	-	100

* 0.1% DMSO is the concentration used for amides solubilization according to the present invention.

** 0.1% ethanol is the concentration used for anandamide and Nabilone solubilization.

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Table 2 - Competitive inhibition of [³H] WIN 55,212-2 binding to RBL-2H3 cell membranes by the amides of the invention and cannabinoids. Incubation was carried out in the presence of 1% DMSO. The values of Inhibitory Concentration 50 (IC₅₀) are expressed as means ± SEM, after 5 the number of experiments reported hereinbelow.

Competitor	IC ₅₀ (nM)	no. of experiments
Nabilone	2.6 ± 1.4	4
Δ ₈ -THC	223 ± 120	6
Cannabidiol	> 1000	3
Anandamide	33 ± 29	5
Example 1	0.4	1
Example 22	18.08	2
Example 25	1.0 ± 0.55	7
Example 27	7.6	1

C) Biological activity assays

The biological activity deriving from CB₂ receptor functional activation and, in particular, the ability of the amides of the invention to inhibit serotonin release, induced by specific stimulus, 10 was evaluated on RBL cultures and compared with cannabinoids of known potency and with anandamide.

The specific binding and/or the functional effects of the amidic derivatives of the invention were evaluated.

For purpose of comparison, the following products were used: among natural cannabinoids, three different isomers of tetrahydrocannabinol (THC): Δ₈-THC, Δ₉-THC and 11-nor-Δ₈-THC-9; among synthetic cannabinoids, 15 Nabilone and WIN 55,212-2, in addition to the CB₁ endogenous ligand.

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anandamide.

Preparation of RBL-2H3 cell cultures

RBL-2H3 cell cultures were grown in flasks (Falcon[®]), at 37°C, 5% CO₂, in the presence of Minimum Essential Eagle's Medium (MEM) and 4 mM glutamine, 100 U/ml penicillin and 20% deactivated fetal calf serum (DFCS).

RBL-2H3 cell sensitization and activation

For each test, the cells were removed from the flasks with PBS, containing 0.5 mM EDTA at pH 7.2, and seeded in wells (96 well plate Falcon[®]) in the presence of RPMI-1640 medium (code 6504, distributed by Sigma) containing 50 mg/l gentamicin and 10% FCS at a density of 100,000 cells/100 µl medium/well. Furthermore, during seeding, said cells were loaded with serotonin by addition of 1 µCi/ml ³H-serotonin (5-hydroxy- triptamine 5HT, 26.4 Ci/mmol, New England Nuclear) and then incubated for 18 hrs (37°C, 5% CO₂). After 18-hr incubation in the presence of ³H- serotonin, the culture medium was removed and the cells were sensitized for 1 hr (37°C, 5% CO₂) in the presence of PIPES (N,N'-bis-(2- ethanesulphonyl)-piperazine, code 3768, Sigma) -buffered saline (100 µl/well), pH 7.1, containing 0.3 µg/ml mouse monoclonal antibody (IgE) against DNP (ADNP).

After removal of the sensitization medium, cells were activated - or not activated (control) - by addition, at 37°C for 155 min, of PIPES (100 µl/well), pH 7.1, containing - or not containing (control) - 0.1 µg/ml human albumin combined with dinitrophenyl (DNP-HSA) and containing a natural or synthetic cannabinoid or an amidic derivative according to the present invention. After incubation, the medium was collected and centrifuged to determine the ³H-serotonin quantity

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released from DNP-HSA-stimulated or not stimulated cells. In parallel, adhered cells were solubilized with 1% Triton X-100 ^(R) in PBS (100 μ l/well) in order to determine the quantity of 3 H-serotonin present in the cells. In both cases, the quantity of 3 H-serotonin was measured by 5 liquid scintillography and radioactivity counting, using a β -counter of Canberra Packard, 1900 TR^{R} .

Addition of cannabinoids or of the derivatives according to the present invention during RBL-2H3 cells activation

It was evaluated the effect of the amidic derivatives of the invention 10 (stock solution in DMSO) and of cannabinoids in the presence or in the absence of anandamide (stock solution in ethanol) on DNP-HSA-induced 3 H-serotonin release in DNP sensitized cells. To this purpose, cannabinoids or the amidic derivatives of the invention, with or without addition of anandamide, were added to the cell activation 15 medium (PIPES \pm DNP-HSA) at the desired concentration. In all cases, the medium was incubated at 37°C for 15 min, while the total final concentration was kept constant (0.2% solvents).

The amides of the invention were solubilized in 0.2% DMSO and 0.1% absolute ethanol, except for N-palmitoyl-ethanolamide, which was 20 solubilized in 1% DMSO and 0.1% ethanol.

Quantification of 3 H-serotonin net release after RBL-2H3 cells activation

3 H-serotonin release in the various samples was calculated according to the following formula:

$$\text{release} = \frac{\text{dpm released}}{(\text{dpm released} + \text{dpm associated with the cells})} \times 100$$

where "dpm" indicates nuclear disintegrations per minute.

The effect of the amides of the invention was expressed as the percentage of ^3H -serotonin net release (i.e. after deduction of the percentage of release in the absence of DNP-HSA) or as the percentage of ^3H -serotonin release inhibition.

5 The results of evaluation tests on the functional effects of cannabinoids and of the amidic derivatives of the invention are shown in Table 2. These data indicate that natural cannabinoids $\Delta 8$ -tetrahydrocannabinol ($\Delta 8$ -THC) and $\Delta 9$ -tetrahydrocannabinol ($\Delta 9$ -THC), the synthetic cannabinoid Nabilone and the labelled receptor agonist 10 WIN 55.212-2 are able to inhibit, in a concentration-dependent way, RBL cells immunogenic activation measured as serotonin release. The compounds described in Examples 1, 25 and 27 can inhibit the effects induced by mast cell activation and exert an effect that is higher than or comparable to the one of cannabinoids.

15 Conversely, anandamide and the compounds described in Examples 19 and 28 are unable to affect serotonin release, like N-(2-hydroxyethyl)-linoleylamide, having two double bonds in the acyl portion and reported in Table 3 by way of comparison. Said data demonstrate a specific structural selectivity in the receptor functional activation: 20 the compounds derived from saturated carboxylic acids are more potent in exerting the functional effects following receptor activation.

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Table 3 - Effect of natural and synthetic cannabinoids and of the amides according to the present invention on serotonin release induced by ADNP/DNP.HSA immunogenic stimulation in RBL-2H3 cells. The compounds were solubilized in 0.1% DMSO and 0.1% ethanol.

Molecules added after sensitization with ADNP	³ H serotonin net release (%)	ED50 (μM)
DNP.HSA-	100	
DNP.HSA+WIN 55,212-2 (50μM)	0	6.4
DNP.HSA+Nabilone (10μM)	0	2.8
DNP.HSA+Δ8-THC (25μM)	0	5
DNP.HSA+Δ9-THC (25μM)	27	5
DNP.HSA+11-nor-Δ8-THC-9 (20μM)	33	15
DNP.HSA+anandamide	100	-
DNP.HSA+Example 1 (3μM)	0	0.6
DNP.HSA+Example 9 (100μM)	0	-
DNP.HSA+Example 10 (100μM)	0	-
DNP.HSA+Example 19 (100μM)	100	-
DNP.HSA+Example 24 (60μM)	67	-
DNP.HSA+Example 25*(10μM)	0	2.6
DNP.HSA+Example 26 (50μM)	21	28
DNP.HSA+Example 27 (7.5μM)	0	3.8
DNP.HSA+Example 28 (100μM)	100	-
DNP.HSA+Example 36 (100μM)	51	-
DNP.HSA+Example 39 (50μM)	68	-
DNP.HSA+N-(2-hydroxyethyl)-linoleylamide (100μM)	100	-

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* At the concentration used (minimum effective concentration), the compound, depending on its solubility limit, was solubilized in 1% DMSO and 0.1% ethanol. At said concentration, DMSO reduces the immunogenic stimulus potency by at least 50%.

5 Co-incubation of anandamide with natural or synthetic cannabinoids and with the derivatives described in Examples 1, 9, 10, 24-27, 36 and 39 reduces the ability of the amides of the invention, as well as of cannabinoids, to inhibit serotonin release, as reported in Table 4.

Table 4 - Anandamide antagonism towards the inhibitory effect of 10 cannabinoids and amidic derivatives of the invention on ADNP/DNP.HSA-induced activation in RBL-2H3 cells.

Molecules added after sensitization with ADNP	3H-serotonin net release inhibition (%)	
	-anandamide	+ anandamide (12.5 μ M)
DNP.HSA+WIN 55.212-2 (30 μ M)	100	36
DNP.HSA+Nabilone (5 μ M)	90	50
DNP.HSA+ Δ 8-THC (25 μ M)	100	n.d.
DNP.HSA+ Δ 9-THC (25 μ M)	73	n.d.
DNP.HSA+11-nor- Δ 8-THC-9 (20 μ M)	67	n.d.
DNP.HSA+Example 1 (3 μ M)	100	39
DNP.HSA+Example 25 (10 μ M)*	100	0
DNP.HSA+Example 26 (50 μ M)	79	48
DNP.HSA+Example 27 (7.5 μ M)	100	46

* At the concentration used (minimum effective concentration), the compound, depending on its solubility limit, was solubilized in 1%

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DMSO and 0.1% ethanol. At said concentration, DMSO reduces the immunogenic stimulus potency by at least 50%.

The data reported in Table 5 show that the co-incubation of anandamide with natural or synthetic cannabinoids and with the amidic derivatives according to the present invention reduces the potency of anandamide, indicating that anandamide causes a competitive antagonism on the peripheral receptor. Therefore, as shown in Table 1, anandamide, though having binding affinity for the peripheral receptor, is not able to induce biological effects and, by competing for the receptor, functionally antagonizes the protective effects of cannabinoids.

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Table 5 - ED₅₀ of cannabinoids and of the amidic derivatives of the invention on the inhibition of ³H-serotonin release induced by ADNP/DNP/HSA stimulation in RBL-2H3 cells, in the presence and in the absence of anandamide.

Molecules	ED50 (μM)	
	-anandamide	+anandamide (12.5 μM)
WIN 55.212-2	6.4	40
Nabilone	2.8	5
Δ 8-THC	5	n.d.
Δ 9-THC	5	n.d.
11-nor-Δ 8-THC-9	15	n.d.
Example 1	0.6	4.0
Example 12	1.1	29.0
Example 23	2.4	10.1
Example 25	2.6	n.d.
Example 26	28	50
Example 27	3.8	9.5

The competitive antagonism of anandamide on the peripheral receptor is exerted by the integral molecule and not by anandamide metabolites, as shown in Table 6.

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Table 6 - ^3H -serotonin release inhibition by WIN 55,212-2 (used at a concentration of 30 μM): antagonistic effect of anandamide vs. the metabolites of the same.

	^3H -serotonin net release inhibition (%)	
	-WIN	+WIN
DNP.HSA	0	69
DNP.HSA + 12.5 μM anandamide	7	15
DNP.HSA + 12.5 μM ethanolamine	6	72
DNP.HSA + 12.5 μM arachidonic acid	4	59
DNP.HSA + 12.5 μM ethanolamide		
+ 12.5 μM arachidonic acid	3	61

As a proof of the close structure-activity relationship, N-(2-hydroxyethyl)-palmitoleylamide (Example 9), like anandamide, shows a binding affinity to the peripheral receptor (as previously shown in Table 1), without exerting biological effects and, by competing for the receptor, functionally antagonizes the protective effects of cannabinoids, as reported in Table 7.

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Table 7 - Inhibition of ^3H -serotonin release in RBL-2H3 cells, induced by ADNP/DNP-HSA stimulation caused by WIN 55,212-2: inhibitory effect of N- (-2-hydroxyethyl)-palmitoleylamide (Example 19)

	Concentration (μM)	^3H -serotonin release inhibition (%)	
		N-(2-hydroxyethyl)-palmitoleylamide (-)	(+)
WIN 55,212-2	5	8	8
	10	12	10
	50	76	24
	70	96	30
	100	98	56

The experimental data of Tables 4 and 8 show that the receptor specificity and the degree of activity are functional to the nature of both the acyl and aminic portions of the amidic derivatives according to the present invention. In fact, the experimental data show a structure- activity specificity as regards the receptor activation. In particular, said data provide evidence that saturated carboxylic acids derivatives are able to exert the most potent functional effects, whereas the presence of several unsaturation points causes the inability to functionally activate the peripheral receptor.

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Table 8 - Inhibitory effect of the amidic derivatives according to the present invention on ^3H -serotonin release induced by ADP/DPN.HSA immunogenic stimulation in RBL-2H3 cells.

Compounds	^3H -serotonin release inhibition %	
	DMSO 0.2%	1%
Example 2	100 (100 μM)	100 (100 μM)
Example 3	100 (100 μM)	100 (100 μM)
Example 4	100 (100 μM)	100 (100 μM)
Example 5	82 (30 μM)	100 (100 μM)
Example 7	93 (30 μM)	100 (100 μM)
Example 9	100 (100 μM)	
Example 12	86 (100 μM)	100 (100 μM)
Example 13	65 (200 μM)	
Example 22	100 (100 μM)	91 (100 μM)
Example 23	60 (60 μM)	91 (100 μM)
Example 24	40 (60 μM)	88 (100 μM)
Example 26	>95% (100 μM)	>95% (100 μM)
Example 29	36 (50 μM)	88 (30 μM)
Example 39	32 (50 μM)	67 (100 μM)

Numbers in parentheses are the concentrations (μM) used for the various compounds in the tests.

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Table 9 - Inhibition of ^3H serotonin release RBL-2H3 immunogenically stimulated. The compounds of the invention were present during the 15 minutes release period. EC50 is the concentration inhibiting by 50% the net release of ^3H serotonin from DNP-HSA-activated RBL-2H3 cells.

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Compound	EC50	
	0.2% DMSO	1% DMSO
Example 2	1.8	5.0
Example 3	1.2	4
Example 4	78	1.05
Example 5	8	7.6
Example 6	30	8.8
Example 7	4.7	
Example 8	18	
Example 11	26	
Example 12	44	1.1
Example 13		32
Example 18	63	
Example 20	6.3	
Example 21	1.5	
Example 22	35	11
Example 23		11
Example 24		7.2
Example 29	42	3.5
Example 30	3.9	
Example 31	7.7	
Example 32	0.73	
Example 33	4.7	
Example 34	13	0.36
Example 35	6.3	9.2
Example 37		15

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The experimental data reported above prove a precise structure-activity correlation in the selective activation of CB2 peripheral receptor, which mediates the non-psychoactive effects of cannabinoids. More precisely, the molecules having a saturated acyclic chain are able 5 to bind and, above all, to functionally activate CB2 peripheral receptor to a significant extent. To the contrary, compounds with more than one double bond in the acyl chain, though having affinity to CB2 receptor, are not able to activate said receptor functionally, thus 10 acting as partial competitive receptor antagonists.

As a proof of this structural specificity, it is known that palmitoylethanolamide, which, as demonstrated by the Applicant, is capable of functionally activating the peripheral receptor, is not able to bind to the central receptor and to mimick the cannabinoid 15 psychoactive action, as instead do compounds having polyunsaturated acyclic chain (W.A. Devane et al., Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor, *Science*, 258:1946-1949, 1992).

Furthermore, the aforementioned experimental results demonstrate that 20 mast cells express the cannabinoid peripheral receptor and that the amidic derivatives according to the present invention behave as competitive agonists of CB2 receptor with an affinity that is higher than, or in any case comparable with the one of natural and synthetic cannabinoids. Moreover, they functionally activate said receptor.

25 Importantly, the Applicant has found that also non-immune cells functionally express cannabinoid peripheral receptor CB2, which was observed for example in cerebellar granule cells, cerebellum, heart and lung.

Said results are extremely interesting from a therapeutic point of view: in fact, the amides of the invention are suitable for the treatment of all diseases for which cannabinoids are notoriously efficacious, acting only on CB2 peripheral receptor and excluding the 5 activity mediated by CB1 central receptor, responsible for untoward effects, such as for example habit and addiction.

In other words, thanks to their specific characteristics, the amidic derivatives of the invention are able to selectively bind and to functionally activate CB2 cannabinoid peripheral receptor. Therefore, 10 they constitute an important therapeutic tool for the treatment of the diseases connected with an anomalous modulation of CB2 cannabinoid peripheral receptor or profiting by the activation of said receptor with consequent negative modulation of cytotoxic or proinflammatory phenomena, or, in any case connected with cytokine, neurokine or 15 enzyme release, or with second messenger activation.

Therefore, the use of said compounds is particularly useful in the preparation of a medicament for the treatment of diseases connected with the modulation of said receptor, such as:

- diseases with nociception alteration;
- 20 - muscular spasm connected with degenerative diseases of the nervous system, such as multiple sclerosis and amyotrophic lateral sclerosis;
- diseases connected with the immune system alteration;
- diseases involving pressure alterations (hypertension) at cardiovascular, pulmonary and ocular levels, glaucoma and cephalgia;
- 25 - nausea, also of iatrogenic nature;
- pathologies caused by biological agents, such as viral (HIV) and bacterial encephalitic meningitis, bacterial meningitis, meningitis by

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cytomegalovirus and AIDS-dementia complex;

- chronic degenerative pathologies such as senile dementia, Alzheimer's and Parkinson's diseases and the associated cachetic symptomatology;

5 - cardiovascular pathologies associated with vascular remodelling, i.e. restenosis after angioplasty including stent application, atherosclerosis and heart attack;

- Chronic airway obstruction including asthma.

In view of their effects, the amidic derivatives according to the 10 present invention are suitable for the manufacture of a medicament for the treatment of human and animal diseases.

For all aforesaid diseases, the systemic administration of the claimed 15 compounds by the oral or parenteral or topical or transdermic ways may be envisaged.

The therapeutically effective dose varies depending on the way of administration and on the method of application as well as on the disease seriousness. It also varies depending on the patient's age, weight and general health conditions. In any case, acceptable 20 therapeutic doses may range from 0.1 to 20 mg/kg/die, preferably from 0.3 to 10 mg/kg/die, over variable periods in any case for at least 30 days.

The pharmaceutical compositions containing as the active principles 25 the amides according to the present invention are inclusive of all formulations containing pharmaceutically acceptable excipients, that are suitable for the administration of the active ingredients in the forms best suited to the disease to be treated and, in any case, rendering the active ingredient as bioavailable as possible.

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In particular, solutions or suspensions for general i.v., s.c. and i.m. administration, solutions for ophthalmic treatment in the form of eyewash, solid or semisolid formulations in the form of inserts, gels and ointments are to be envisaged. As concerns oral formulations, 5 granular powders, tablets, pills and capsules will be preferred.

As concerns dermic and transdermic administration, creams, ointments, gels and plasters, where the active ingredient may be included in slow-releasing microspheres, will be preferred.

The following examples, reported by way of indication, not of 10 limitation, of the present invention, are meant to provide evidence of possible industrial applications of the invention.

EXAMPLE 40

Vials for injection

Every vial contains:

15	Compound of Example 9	20	mg
	Mannitol	12	mg
	Sodium metasulphite	1.3	mg
	Benzyl alcohol	80	mg
	Propylene glycol	400	mg
20	Sodium hydroxide	q.s. to pH 7	
	Water for injectable formulations	q.s. to 2 ml	

EXAMPLE 41

Tablets

Every tablet contains:

25	Compound of Example 9	50	mg
	Dibasic dihydrated calcium phosphate	135.2	mg
	Microgranular cellulose	36	mg

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	Maize starch	7.2 mg
	Magnesium stearate	1.8 mg
	Hydrogenated vegetable oil	1.2 mg
	Precipitated silica	0.6 mg
5	Hydroxypropylene ethylcellulose	4.7 mg
	Titanium dioxide	0.3 mg

EXAMPLE 42

Eyewash

Every bottle contains:

10	Compound as per Example 27	25 mg
	Borax	15 mg
	Boric acid	75 mg
	Polysorbate 80	15 mg
	Lactose	80 mg
15	Phenol	3.9 mg
	Disodium edetate	5 mg
	Water for injectable formulation	q.s. to 5 ml

EXAMPLE 43

Soft capsules

20 Every capsule contains:

Compound of Example 1	100 mg
Excipient: peanut oil O.P.	100 mg

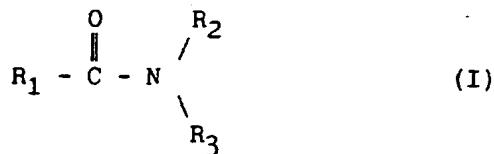
Composition of the capsule:

gelatin O.P., glycerin O.P., natural dye E12.

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CLAIMS

1 1. Use as the active principle in the preparation of pharmaceutical
 2 compositions for the treatment of human and animal diseases connected
 3 with the modulation of CB2 cannabinoid peripheral receptor, of at
 4 least an amide of formula (I):



5 wherein R_2 and R_3 belong to one of the following classes:

6 A) R_2 is the residue of a linear or branched hydroxyalkyl containing 1
 7 to 20 carbon atoms, optionally substituted with one or more phenyl
 8 groups; R_3 is H, CH_3 or = R_2 ;

9 B) R_2 is the residue of an alkylene-hydroxyphenyl, the aromatic ring
 10 being optionally substituted with one or more $-\text{OH}$ and/or $-\text{OCH}_3$ groups
 11 and the linear or branched alkylene chain containing 1 to 20 carbon
 12 atoms;

13 R_3 is H, CH_3 or = R_2 ;

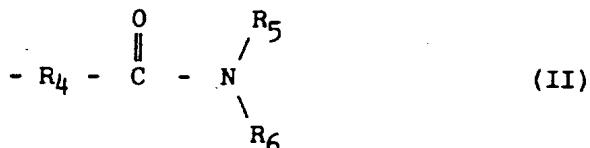
14 C) R_2 and R_3 , with the nitrogen atom to which said two substituents
 15 are linked, form the residue of a cyclic aminoether containing 5 to 7
 16 carbon atoms, optionally substituted with linear or branched alkyl
 17 groups;

18 in the above classes, the alcoholic function $-\text{OH}$ can be optionally
 19 functionalized to give $-\text{OX}$, where X can be an alkyl, an acyl, an O-
 20 phosphate, the amiacyl of a bicarboxylic acid, an alkyl-sulphonate, a
 21 sulphate, a dialkylaminoacyl or an aminoacyl, X being optionally
 22 salified with monovalent or bivalent inorganic ions;

23 and wherein R_1 is:

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24 1) a linear or branched hydrocarbon radical containing 9 to 23 carbon
25 atoms, either saturated or containing one double bond, optionally
26 substituted with one or more -OH groups;
27 2) a residue of formula (II):



28 where R_4 is a linear or branched hydrocarbon radical containing 8 to
29 22 carbon atoms, either saturated or containing one double bond,
30 optionally substituted with one or more -OH groups;
31 R_5 and R_6 being defined as R_2 and R_3 , respectively.

1 2. The use according to claim 1, characterised in that, when R₁
2 belongs to class (1), it is a hydrocarbon radical containing 11 to 17
3 carbon atoms.

1 3. The use according to claim 1, characterised in that, when R_1
2 belongs to class (2), R_4 is a hydrocarbon radical containing 10 to 16
3 carbon atoms.

1 4. The use according to claim 1, characterised in that, when R₁
2 belongs to class (1), it forms, with the adjacent carbonyl, the acyl
3 of a monocarboxylic acid selected from the group consisting of lauric,
4 myristic, palmitic, stearic, palmitoleic, oleic and ω -hydroxypalmitic
5 acids.

1 5. The use according to claim 1, characterised in that, when R_1
2 belongs to class (2), R_4 forms, with the two adjacent carbonyls, the
3 acyl of traumatic acid.

1 6. The use according to claim 1, characterised in that, when R_2 and R_3
2 belong to class (A), R_2 forms, with the nitrogen atom to which it is

3 linked, the residue of monoethanolamine, diethanolamine, 2-hydroxy-
4 propyl-amine or di-(2-hydroxy-propyl)-amine.

1 7. The use according to claim 1, characterised in that, when R₂ and R₃
2 belong to class (B), R₂ forms, with the nitrogen atom to which it is
3 linked, the residue of tyramine or 4-hydroxy-3-methoxy-benzylamine.

1 8. The use according to claim 1, characterised in that, when R₂ and/or
2 R₃ belong to class (C), R₂ and R₃ form, with the nitrogen atom to
3 which it is linked, the residue of morpholine.

1 9. The use according to claim 1, characterised in that, when X is an
2 alkyl residue, it is methyl or ethyl.

1 10. The use according to claim 1, characterised in that, when X is an
2 acyl, it is -CO-CH₃ or -CO-Ph.

1 11. The use according to claim 1, characterised in that, when X is an
2 O-phosphate, it is -PO₃H₂ or -PO₂H-O-CH₂-CH(OH)-CH₂-OH.

1 12. The use according to claim 1, characterised in that, when X is the
2 emiacetyl of a dicarboxylic acid, it is -CO-CH₂-CH₂-COOH or
3 -CO-(CH₂)₃-COOH.

1 13. The use according to claim 1, characterised in that, when X is an
2 alkyl-sulphonate, it is -SO₂-CH₃, -SO₂-C₆H₅ or -SO₂-C₆H₄-CH₃.

1 14. The use according to claim 1, characterised in that, when X is a
2 dialkylaminoacyl, it is -CO-CH₂-N(CH₃)₂.

1 15. The use according to claim 1, characterised in that, when X is an
2 aminoacyl, it is -CO-CH(W)-NH₂, wherein W is the lateral chain of a
3 natural aminoacid.

1 16. The use according to claim 1, characterised in that X is salified
2 with the ions of K, Na, Mg or Ca.

1 17. The use according to claim 1, characterised in that said active

2 principle is administered at a dosage ranging from 0.1 to 20
3 mg/kg/die, for at least 30 days.

1 18. The use according to claim 17, characterised in that said active
2 principle is administered at a dosage ranging from 0.3 to 10
3 mg/kg/die.

1 19. The use according to claim 1, characterised in that said
2 pharmaceutical compositions are orally, parenterally, topically or
3 transdermically administered.

1 20. The use according to claim 19, characterised in that said
2 pharmaceutical compositions are parenterally administered by i.v.,
3 s.c. or i.m. routes, in the form of solutions or suspensions.

1 21. The use according to claim 19, characterised in that said
2 pharmaceutical compositions are topically administered in the form of
3 eyewash for ophtalmic use, or in solid or semisolid formulations,
4 insert, gel or ointment.

1 22. The use according to claim 19, characterised in that said
2 pharmaceutical compositions are orally administered in the form of
3 granular powders, tablets, pills or capsules.

1 23. The use according to claim 19, characterised in that said
2 pharmaceutical compositions are topically or transdermically
3 administered, in the form of cream, ointment, gel or plaster, said
4 active ingredient being optionally included in slow-releasing
5 microspheres.

1 24. The use according to claim 1, characterised in that said diseases
2 are diseases with nociception alteration.

1 25. The use according to claim 1, characterised in that said diseases
2 are muscular spasm connected with degenerative diseases of the nervous

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3 system selected from multiple sclerosis and amyotrophic lateral
4 sclerosis.

1 26. The use according to claim 1, characterised in that said diseases
2 are pathologies connected with the immune system alteration.

1 27. The use according to claim 1, characterised in that said diseases
2 are pathologies involving pressure alterations at cardiovascular,
3 polumnary or ocular level.

1 28. The use according to claim 27, characterised in that said diseases
2 involving pressure alterations are glaucoma or cephalea.

1 29. The use according to claim 1, characterised in that said disease
2 is nausea.

1 30. The use according to claim 1, characterised in that said diseases
2 are pathologies caused by biological agents, selected from the group
3 consisting of viral (HIV) and bacterial encephalitic meningitis,
4 bacterial meningitis, meningitis by cytomegalovirus and AIDS-dementia
5 complex.

1 31. The use according to claim 1, characterised in that said diseases
2 are chronic degenerative pathologies selected from the group
3 consisting of senile dementia, Alzheimer's and Parkinson's diseases
4 and the associated cachetic symptomatology.

1 32. The use according to claim 1, characterised in that said diseases
2 are cardiovascular pathologies associated with vascular remodelling,
3 atherosclerosis or heart attack.

1 33. The use according to claim 1, characterised in that said disease
2 is chronic airway obstruction.

1/2

A B C



FIG. 1

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FIG. 2

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/EP95/04927 (22) International Filing Date: 13 December 1995 (13.12.95) (30) Priority Data: MI94A002512 14 December 1994 (14.12.94) IT		(81) Designated States: AL, AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 15 August 1996 (15.08.96)	
(71) Applicant (for all designated States except US): LIFEGROUP S.P.A. [IT/IT]; Viale Tre Venezie, 44/46, I-35043 Monselice (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): DELLA VALLE, Francesco [IT/IT]; Via Cerato, 14, I-35122 Padova (IT). LEON, Alberta [IT/IT]; Piazza Napoli, 19, I-35142 Padova (IT). MARCOLONGO, Gabriele [IT/IT]; Strade Interne, 5 - Terra, I-35020 Carrara San Giorgio (IT). LORENZI, Silvana [IT/IT]; Piazza Napoli, 17, I-35142 Padova (IT). (74) Agent: GERVASI, Gemma; Notarbartolo & Gervasi S.r.l., Viale Bianca Maria, 33, I-20122 Milan (IT).			
(54) Title: USE OF MONO AND BICARBOXYLIC ACID AMIDES FOR THE MANUFACTURE OF A MEDICAMENT ACTIVE AT THE PERIPHERAL CANNABINOID RECEPTOR (57) Abstract <p>The following description concerns the therapeutic use of amide derivatives of mono and dicarboxylic acids with aminoalcohols or aminoethers in the manufacture of a medicament for the treatment of diseases connected with the modulation of the cannabinoid peripheral receptor, said amide derivatives being selectively active on said receptor.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04927

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/16 A61K31/165 A61K31/535

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO,A,95 25509 (LIFEGROUP) 28 September 1995 cited in the application see claims 1,7,14,20 see page 27, line 15 - line 27 ---	1-33
X, P	PROC.NATL.ACAD.SCI.USA, vol. 92, no. 8, 11 April 1995, pages 3376-80, XP002005694 "Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide" see page 3379 - page 3380 ---	1-33 -/-

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04927

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EUR.J.CLIN.PHARMACOL., vol. 7, no. 6, 1974, pages 415-419, XP000573471 "Prophylactic efficacy of N-2-Hydroxyethyl palmitate (Impulsin) in acute respiratory tract infections" see the whole document ---	1-33
A	EP,A,0 550 008 (LIFEGROUP) 7 July 1993 cited in the application see the whole document ---	1-33
A	EP,A,0 550 006 (LIFEGROUP) 7 July 1993 cited in the application see the whole document ---	1-33
A	NATURE, vol. 365, no. 6441, 1993, pages 61-65, XP002005676 "Molecular characterization of a peripheral receptor for cannabinoids" see the whole document -----	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/04927

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9525509	28-09-95	AU-B- 2109095	09-10-95
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AMENDED CLAIMS

[received by the International Bureau on 15 July 1996 (15.07.96);
original claims 30 and 31 amended; remaining claims unchanged (1 page)]

3 system selected from multiple sclerosis and amyotrophic lateral
4 sclerosis.

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1 27. The use according to claim 1, characterised in that said diseases
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1 28. The use according to claim 27, characterised in that said diseases
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1 30. The use according to claim 1, characterised in that said diseases
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3 consisting of viral (HIV) and bacterial encephalitic meningitis,
4 bacterial meningitis and meningitis by cytomegalovirus.

1 31. The use according to claim 1, characterised in that said disease
2 is cachetic symptomatology associated with chronic degenerative
3 pathologies selected from the group consisting of senile dementia,
4 Alzheimer's and Parkinson's diseases.

1 32. The use according to claim 1, characterised in that said diseases
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3 atherosclerosis or heart attack.

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